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Total Organic Iodine Quantification and Occurrence in Drinking Water, and Toxicity Assessment of Iodinated Disinfection By-Products

Rassil El Sayess

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Total Organic Iodine Quantification and Occurrence in Drinking Water, and Toxicity
Assessment of Iodinated Disinfection By-Products

A Dissertation Presented

by

RASSIL EL SAYESS

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

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To my husband, Scott, and to our little turtle, Yasmina
and forever, with love, to my grandfather, Abu Anis

الى زوجي، سكوت، والى سلحفاتنا الصّغيرة، ياسمينه
والى الأبد، مع الحب، الى جدي، أبو أنيس

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To my mom, for her unwavering support throughout the years, her every day patience in listening to my ordeals and complaints, and her infinite love. Further, both my birth and acquired families have been a strong source of support and encouragement, and I feel very fortunate to have them in my life.

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ABSTRACT

TOTAL ORGANIC IODINE QUANTIFICATION AND OCCURRENCE IN DRINKING WATER, AND TOXICITY ASSESSMENT OF IODINATED DISINFECTION BY-PRODUCTS

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The focus of this work has been placed is on iodinated DBPs (I-DBPs), measured using total organic iodine (TOI), a surrogate measure of iodinated organics. This is due to the growing toxicity literature that places I-DBPs among the most toxic of all DBPs. To measure TOI in water, a new method was developed. This method combines adsorption, combustion, and trapping of combustion products, with an offline inductively coupled plasma/mass spectrometer (ICP-MS) for iodide detection. Three factors were varied across two levels each in order to optimize the method. The chosen method used a sample pH of less than 1 prior to adsorption, a solution of 2% tetramethyl ammonium hydroxide (TMAH) in the trap solution, and a TMAH wash solution for the ICP-MS.

The method was then used to quantify TOI in raw and treated waters in three water treatment plants in the Northeast US over a period of fifteen months across different treatment plants and in different locations within the same systems. The results showed that there was substantial inter-monthly variability in TOI where values cluster high or low for months at a time. There was no change in TOI concentration upon treatment, suggesting that the TOI components may have shifted between the raw and treated waters. The results of a multivariate regression showed that dissolved organic carbon, specific UV₂₅₄ absorbance, combined residual chlorine, and pH were all correlated with TOI concentration. These parameters were then used to fit a predictive model for TOI formation in water.

Expanding on the current literature on the toxicological profile of I-DBPs, the impact of six I-DBPs on healthy human colon epithelial cells, CCD 841 CoN, was tested. The rank order for cytotoxicity of the I-DBPs was found to be iodoacetic acid >iodoacetamide >bromiodoacetamide >chloriodoacetamide> bromiodoacetic acid ≈

diiodoacetic acid. Iodoacetamide was 3.5 times more cytotoxic than bromoiodoacetamide, which in turn was 2.7 times more cytotoxic than chloroiodoacetamide. For both dihaloacids, the cytotoxicity was less than 1% of that of the monohaloacid. Apart from iodoacetic acid, the nitrogenous I-DBPs evaluated in this study proved to be more cytotoxic than the carbonaceous I-DBPs.

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CHAPTER 1

OVERVIEW AND INTRODUCTION

Chlorination of drinking water is considered one of the most important advancements in public health in the twentieth century, having led to a substantial decrease in water-borne diseases in the United States (CDC, 1999). Microorganisms are effectively inactivated in drinking water through the use of powerful oxidants such as chlorine, chlorine dioxide, and chloramines. The non-selective nature of these chemicals also leads to the oxidation of natural organic matter (NOM) and bromide/iodide that are naturally present in source waters, and further yields halogenated disinfection by-products (DBPs). In 1974, trihalomethanes were the first DBPs identified in chlorinated waters (Rook, 1974). Four decades later, more than 600 DBPs have been identified in the literature (Richardson et al., 2008). The parameter total organic halogen (TOX) has been used as a surrogate measure of the sum of all DBPs in a water sample (Li et al., 2002; Reckhow et al., 1990; Richardson, 2003). Despite the hundreds of DBPs that have been identified so far, 50% of the individual compounds that make up TOX in chlorinated waters remain unidentified, while the percentage is even higher in chloraminated waters (Christman et al., 1983; Diehl et al., 2000; Hua and Reckhow, 2006; Kanniganti et al., 1992; Krasner et al., 1989; Miller et al., 1983; Reckhow and Singer, 1984; Richardson, 2003).

Epidemiological studies have reported a positive association between exposure to DBPs (particularly trihalomethanes) in chlorinated drinking water to an increased risk of bladder, colon, and rectum cancers (Bove et al., 2007; Bull et al. 1995; Cantor et al., 2010; Costet et al., 2011; King and Marrett, 1996; King et al., 2000; Koivusalo et al.,

1997; McGeehin et al., 1993; Morris et al., 1992; Rahman et al., 2010; Villanueva et al., 2004; Villanueva et al., 2007). This raised an important public health issue and led to the regulation of eleven DBPs under the Stage 1 and Stage 2 Disinfectant/Disinfection Byproduct (D/DBP) Rules (United States Environmental Protection Agency (USEPA), 1999; 2003). The use of chloramine as a final disinfectant leads to a lower formation of regulated DBPs (Krasner et al., 1989; Zhang et al., 2000). This prompted a shift from the use of free chlorine to chloramine as a final disinfectant in many US water utilities (Krasner et al., 1989; Seidel et al., 2005; USEPA, 2012; Zhang et al., 2000). Later studies showed that even though chloramination produced less of the regulated DBPs, it led to the formation of unregulated, and concerning DBPs (Krasner et al., 2006; Kristiana et al., 2009; Plewa et al., 2004b; Richardson et al., 2008; Weinberg et al., 2002;).

Recent toxicity literature has shown that as a group, iodinated-DBPs (I-DBPs) are more cyto- and genotoxic than brominated-DBPs, which are in turn more cyto- and genotoxic than chlorinated-DBPs in Chinese Hamster Ovary (CHO) cells (Hunter and Tugman, 1996; Plewa et al., 2008; Plewa et al., 2010; Richardson et al., 2008). Therefore, breaking down the parameter TOX into its individual halogens can be extremely informative. Similar to the use of TOX, total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) have been adopted as surrogates for total chlorinated, brominated, and iodinated organics in water sources, respectively (Hua and Reckhow, 2006; Hua and Reckhow, 2007; Hua et al., 2006; Kristiana et al., 2009; Oleksy-Frenzel et al., 2000). The focus of this work is on TOI partly because of the growing toxicity literature that places I-DBPs among the most toxic of all halogenated DBPs. Currently, there is a need for a method that is rapid and sensitive and can capture a

wide range of iodinated organics in water. The lack of a good method had contributed to a void in understanding the behavior of TOI in real water systems. To be able to fill this void, a method for TOI measurement in water that meets those objectives should be developed. In this work, an improved TOI method is developed that builds on previous methods, yet is optimized to offer the highest sensitivity and applicability. With the availability of such a method, we will be able to accurately quantify and characterize TOI in raw and disinfected water samples. This enables us to explore the occurrence, magnitude, and seasonality of TOI in real water systems. Having a large scale understanding of TOI in real water systems makes it possible to relate TOI to the physical and chemical parameters that are routinely measured in treated waters. The ultimate goal of this line of work would be to better estimate TOI occurrence in treated water using these parameters in the absence of advanced analytical techniques.

The foundation of most of the aforementioned toxicity literature on DBPs in general and I-DBPs in particular has been built on results from CHO cells. Since natural heterogeneity warrants that the effect of the same toxicant will vary between species, the responses observed in CHO cells cannot necessarily be extended to humans. This gives rise to the need to study human cell lines that can be directly related to the available epidemiological evidence. In this study, we tested the impact of six I-DBPs on healthy human colon epithelial cells. Not only can this work serve to complement the existing literature on CHO cells, it should also be a starting point and a building block for an extensive toxicity study of I-DBPs and other halogenated DBPs using this cell line. The hope is that this cell culture can be used to guide future water treatment approaches to minimize the public health risk associated with DBPs exposure through drinking water.

CHAPTER 2

AN IMPROVED METHOD FOR TOTAL ORGANIC IODINE IN DRINKING WATER¹

2.1 Introduction

The use of chlorine as a chemical disinfectant to purify drinking water is one of the most important public health achievements of the twentieth century, having led to a significant decrease in water-borne diseases in the U.S. (Center for Disease Control and Prevention, 1999). However, the non-selective properties of powerful oxidants such as chlorine, ozone, and chlorine dioxide means that they not only act on pathogens, but they also oxidize natural organic matter (NOM), bromide, and iodide naturally present in source waters to form halogenated disinfection by-products (DBPs). Moreover, organic-bound sources of iodine in raw waters may participate as well. For example, iodinated X-ray contrast media (ICM) commonly used in medical imaging are poorly removed in wastewater treatment and as a result, they have been detected in rivers and streams (Carballa et al., 2004; Oleksy-Frenzel et al., 2000; Putschew et al., 2001; Putschew and Jekel, 2006), as well as in groundwater and drinking water (Drewes et al., 2001; Hirsch et al., 2000; Putschew et al., 2001; Sacher et al., 2001; Ternes et al., 2003; Schittko et al., 2004). These compounds have the ability to react with the added disinfectants during treatment, releasing iodine and forming iodinated-DBPs (I-DBPs).

The complex nature of organic matter in chlorinated and chloraminated water leads to the formation of a large number of halogenated-DBPs that cannot all be individually identified and quantified. Measures of total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) have been commonly adopted as

¹ Modified from originally published version (Sayess, R. & Reckhow, D. A., 2017. Improved method for total organic iodine in drinking water. *Water Res.*, 108, 250-259.)

surrogates for total chlorinated, brominated, and iodinated organics in water sources, respectively (Oleksy-Frenzel et al., 2000; Hua and Reckhow, 2006; Hua and Reckhow, 2006; Hua et al., 2007; Kristiana et al., 2009), in a similar manner to the use of total organic halide, or TOX, as a surrogate for the sum of all halogenated organics (Li et al., 2002; Reckhow et al., 1990; Richardson, 2003). Since I-DBPs are of particular concern due to their elevated cyto- and geno-toxicity compared to their brominated and chlorinated analogs (Hunter and Tugman, 1996; Plewa et al., 2008; Plewa et al., 2010; Richardson et al., 2008), there is increasing interest in the measurement and tracking of TOI as a surrogate for the total amount of iodinated organics in disinfected water.

The most widely used methods for TOI measurement entail the adsorption of the organic iodine in an acidified water sample (pH~2) onto activated carbon, pyrolysis of the organic iodine to form hydrogen iodide (HI) off-gas, and collection of the HI into an aliquot of water (trap solution). For iodide detection and separation from the aliquot of water, the use of either offline ion chromatography (IC; Hua and Reckhow, 2006; Kristiana et al., 2009; Oleksy-Frenzel et al., 2000) or offline ultra-performance liquid chromatography/electrospray ionization-mass spectrometry (UPLC/ESI-MS; Ding and Zhang, 2009; Gong and Zhang, 2015; Pan and Zhang, 2013) have been used. Both detection methods have drawbacks. The standard IC method has poor sensitivity for iodide (method detection limit of about 100 µg/L as I). Although iodide sensitivity can be improved by using a proper ratio of sample volume to trap solution volume (Kristiana et al., 2009), an IC run takes about 25 minutes per sample, precluding its routine analysis. The UPLC/ESI-MS detection method showed improvements over standard IC with faster analysis, better sensitivity (method detection limit of 3.7 µg/L as I), and higher

chromatographic resolution. However, it failed to detect iodide peaks for iopromide, designated by the authors as a representative of ICM compounds (Pan and Zhang, 2013). This raises questions regarding the use of UPLC/ESI-MS for the detection of some of the major ICM compounds. Considering that the presence of ICM compounds in raw water may lead to the formation of I-DBPs upon disinfection, the chosen method has to be able to detect these compounds.

The inductively coupled plasma – mass spectrophotometer (ICP-MS) has superior sensitivity for iodine (I) in comparison to other detection techniques (Takaku et al., 1995). For that reason, many studies have utilized ICP-MS in various natural waters after separation of organic and inorganic halogens with high performance size exclusion (Gilfedder et al., 2010; Gilfedder et al., 2011; Heumann et al., 1998; Radlinger and Heumann, 2000; Radlinger and Heumann, 1997). The objective of this study was to develop and optimize a method that takes advantage of the superior sensitivity of ICP-MS and therefore overcomes the limitations of previous methods. The proposed method entails adsorption, combustion, and trapping, with offline ICP-MS for iodide detection. Key to any TOI method is the ability to achieve near complete recovery of iodinated compound that represent those expected to form as DBPs, while exhibiting high rejection of inorganic forms of iodine (e.g., iodide, triiodide, iodate).

2.2 Materials and Methods

2.2.1 Instrumentation

The instrumentation for TOI analysis includes adsorption and combustion units and an off-line ICP-MS. The adsorption systems used were either an EFU 1700 Filtration

Unit (Euroglas BV, Delft, The Netherlands) or an XPREP-A6 (Trace Elemental Instruments, Delft, The Netherlands), both equipped with pressurized sample reservoirs and granular activated carbon (GAC) adsorption columns (CPI International, Santa Rosa, CA). The ECS 1200 combustion system (Euroglas BV, Delft, The Netherlands) includes a combustion glass tube, a boat sampler, a motor-driven boat sampler, a furnace, sulfuric acid scrubbers, a gas bubbler/diffuser and trap, and an oxygen gas supply (99.99% high-purity grade). The ICP-MS (Perkin Elmer Elan 9000) was used for measuring iodide concentration in the trap solution. Argon (99.99% high-purity grade) was used as the carrier and reaction gas.

2.2.2 Chemicals and reagents

Ultra-pure water was obtained by filtering de-ionized water with a resistivity greater than 18.3 MΩ.cm (Billerica, MA) and used in preparing procedural calibration standard solutions, laboratory reagent blanks, model compound solutions, and the ICP-MS wash solutions. Potassium iodide (KI), sodium iodide (NaI), sodium iodate (NaIO₃), and nitric acid (HNO₃; 70%, Trace Metal grade, Certified ACS) were purchased from Fisher Scientific. Tetramethylammonium hydroxide (TMAH; 25% W/W aqueous solution, Electronic Grade 99.999%) was obtained from Alfa Aesar. The model compounds iodoacetic Acid (IAA, 98%), 2-hydroxy-3-iodo-5-nitropyridine (97%), and 3-iodo-4-methylbenzoic acid (97%) were obtained from Aldrich Chemical Company, while bromiodoacetic acid (BIAA, 90+%) and triiodoacetic Acid (TIAA, 90%) were purchased from CanSyn Chem Corp and Toronto Research Chemical, respectively. The three ICM model compounds iopromide, diatrizoic acid, and iopamidol were obtained from European Pharmacopeia, Sigma Chemical Company, and USP, respectively.

2.2.3 Experimental design

The new proposed method builds on some of the previous methods presented in Table 2.1. During method development, three experimental factors were varied across two levels each to find the optimal combination of levels, hereafter referred to as “*treatment*”, for TOI recovery. The factors include: (Factor 1) the pH of the solution prior to GAC adsorption; (Factor 2) the amount of TMAH added to the iodide trap solution; and (Factor 3) the choice of ICP-MS wash. A detailed description of the entire process along with the reasons behind choosing the three factors is presented below.

Table 2.1: Summary of methods for TOX and TOX species

Study	Objective	pH of sample to be adsorbed	Nitrate wash	Detection	Reported concentration
Oleksy-Frenzel et al., 2000	TOX speciation	2	Yes	IC	Cl ⁻ equivalent
Hua and Reckhow, 2006	TOX speciation	2	Yes	IC	Cl ⁻ equivalent
Kristiana et al., 2009	TOX speciation	2	Information not available	IC	Cl ⁻ equivalent
Pan and Zhang, 2013	TOI	2	Yes	UPLC/ESI-MS/MS	TOI
This study	TOI	2 or <1	No	ICP-MS	TOI

2.3.3.1. Factor 1 (F1) – sample pH during adsorption

Both adsorption efficiency and compound stability are affected by pH. In these experiments, the water sample to be analyzed was acidified using HNO₃ to either pH = 2 ± 0.2 (Level 1) or pH < 1 (Level 2). During our experimental runs, the range of pH < 1 corresponded to a pH range between 0.76 and 0.99. Decreasing the pH shifts speciation of acidic compounds to protonated forms which are more readily adsorbed by activated

carbon. Fifty mL of the acidified sample is then applied at a flow rate of 3 mL/min onto two consecutive GAC columns (*Col#1 and Col #2*). The two GAC columns are then sequentially placed in a ceramic boat that is introduced into a 1000 °C oven to be combusted in the presence of oxygen gas for 10 minutes. Upon combustion, the organic iodine is released as HI in the gas phase that gets carried through sulfuric acid scrubbers and into a custom-made absorber cell that contains about 15 mL of ultra-pure water (the trap solution). At the end of the trapping cycle, the trap solution is adjusted to 20 mL by rinsing out the walls of the absorber cell.

2.2.3.2. Factor 2 (F2) – composition of the trap solution

The trap solution should be selected to retain all gaseous HI and keep the iodine in a form that is effective for subsequent analysis by ICP-MS. An important consideration is that iodine is known for its volatile nature and its tendency to persist on interior surfaces in analytical instruments, and undergo slow release during analysis of subsequent samples. These “memory effects” can introduce substantial error, particularly in acidic solutions (Takaku et al., 1995). Often, memory effects can be partly mitigated by long wash periods between samples, but this is far from an ideal solution. To prevent this problem, previous studies have suggested preparing the samples in an alkaline solution to improve iodine retention and signal stability, and to reduce the memory effect (Baumann, 1990; Gélinas et al., 1998; Muramatsu and Wedepohl, 1998; Takaku et al., 1995; Vanhoe and Van Allemeersch, 1993). The choice of TMAH in particular as the alkaline solution in this study was motivated by its small matrix effect and good stability with the ICP (Takaku et al., 1995). In addition, its use results in high pH values without increasing the salt concentration; a problem that can occur with the use of sodium

hydroxide (NaOH) or potassium hydroxide (KOH) (Shetaya et al., 2012). This lowers the possibility of salt deposition in the ICP torch and nebulizer. In this study, TMAH at 0.1% v/v (Level 1; equivalent to pH~10) or 2% v/v (Level 2; equivalent to pH~12) was added to the 20 mL trap solution, then analyzed offline using the ICP-MS.

2.2.3.3. Factor 3 (F3) – composition of ICP-MS wash

For the choice of the ICP-MS wash solution, the literature varies in terms of what the best option is for iodine measurement. Various studies have used a range of percentages of acidic (mainly HNO₃) or basic (KOH, ammonium hydroxide, and TMAH) ICP-MS washes (Bu et al., 2003; Hansen et al., 2011; Muramatsu and Wedepohl, 1998; Patriarca et al., 1999). Previous studies looking at iodine in a range of geological reference materials used diluted HNO₃ as the ICP-MS wash and were able to produce stable and reproducible results (Michel and Villemant, 2003; Schnetger and Muramatsu, 1996; Schnetger et al., 1998). Other studies that used TMAH as a sample solvent also used it as the wash solution at varying percentages and also produced sensitive and stable results (Mesko et al., 2010; Muramatsu and Wedepohl, 1998). In this study, we varied the wash solution between HNO₃ at 2% v/v (Level 1) and TMAH at 0.1% v/v (Level 2).

2.2.4 Experimental runs

TOI recovery across the three factors and each of their two levels is examined for a variety of samples to determine the effectiveness of each *treatment*. All eight *treatments* (Table 2.2) were tested in a full factorial design for eight different organic model compounds, two inorganic compounds, and a series of field water samples. These

different waters are described below. To account for any background iodine, an ultra-pure Milli-Q blank was analyzed along with each of the tested waters.

Table 2.2: An overview of the experimental runs for every sample without duplication/triplication

<i>Treatment</i>	Factor 1: pH of adsorbed sample	Factor 2: Percentage (v/v) TMAH added	Factor 3: ICP-MS wash
1	~2	0.1	HNO ₃ (2%)
2	<1	0.1	TMAH (0.1%)
3	~2	2	HNO ₃ (2%)
4	<1	2	TMAH (0.1%)
5	~2	0.1	TMAH (0.1%)
6	<1	0.1	HNO ₃ (2%)
7	~2	2	TMAH (0.1%)
8	<1	2	HNO ₃ (2%)

2.2.4.1. Model organic compounds

Eight organic iodinated model compounds prepared at known concentrations were examined in duplicate under each of the 8 *treatments* to provide a direct measure of TOI recovery (Table 2.3). The compounds include three ICM compounds (iopromide, diatrizoic acid, and iopamidol), three iodinated DBPs (IAA, BIAA, and TIAA), in addition to 2-hydroxy-3-iodo-5-nitropyridine, and 3-iodo-4-methylbenzoic acid. Zhang and Minear (2002) used a quantitative structure-activity relationship (QSAR) to estimate the decomposition rate constant for TIAA of 50 day⁻¹ (corresponding to a half-life of 0.014 days) at a pH of 7 and a temperature of 23 °C. Therefore, we assumed that the TIAA had entirely decomposed to iodoform at the time of analysis.

Table 2.3: Summary of the inorganic and organic iodinated compounds used during method development

Compound Name	Molecular Formula	Molecular Weight (g.mol ⁻¹)	pKa	Theoretical prepared concentration (µg.L ⁻¹ as I)
Iopromide	C ₁₈ H ₂₄ I ₃ N ₃ O ₈	791.11	10.62 ± 0.7	213.2
Diatrizoic acid	C ₁₁ H ₉ I ₃ N ₂ O ₄	613.91	0.92 ± 0.1	152.3
Iopamidol	C ₁₈ H ₂₂ I ₃ N ₃ O ₈	777.08	10.87 ± 0.46	190.4
Iodoacetic acid	C ₂ H ₃ IO ₂	185.95	3.18 ± 0.1	126.9
Bromoiodoacetic acid	C ₂ H ₂ BrIO ₂	264.84	1.67 ± 0.1	126.9
¹ Triiodoacetic acid	C ₂ HI ₃ O ₂	437.74 (393.73 for iodoform)	1.29 ± 0.41	114.2
2-hydroxy-3-iodo-5-nitropyridine	C ₅ H ₃ IN ₂ O ₃	265.99	6.62 ± 0.1	126.9
3-iodo-4-methylbenzoic acid	IC ₆ H ₃ (CH ₃)CO ₂ H	278.04	4.02 ± 0.1	126.9
Iodide	I ⁻	126.90	8.49	31.7 and 63.4
Iodate	IO ₃ ⁻	174.90	0.75	13.7 and 68.7

2.2.4.2. Model inorganic compounds

Recovery of iodine by the TOI method was also determined for inorganic iodinated compounds to determine the extent of interference that might be caused by these compounds. For all 8 *treatments*, two inorganic compounds at two concentrations were tested in triplicates: NaI at 31.7 and 63.4 µg/L as I and NaIO₃ at 13.7 and 68.7 µg/L as I.

After the full analysis of all 8 *treatments* was completed and the best *treatment* identified, solutions of KI and NaIO₃ at three concentrations (50.8, 25.4, and 5.1 µg/L as I), and an equi-molar mix of both compounds at four concentrations (50.8, 25.4, and 5.1 µg/L as I) were analyzed in triplicate using that method. This extra step was taken to determine the behavior of iodide and iodate species across a larger range of concentrations for the chosen method.

2.2.4.3. Field water samples

Three raw and nine treated water samples (from two river sources and three water treatment plants in the Northeastern US) were collected during two summer months in 2015 for analysis. Ascorbic acid was added at 40 mg/L to each of the samples as a quenching agent. A brief summary of the physical and chemical characteristics of the 12 water samples during the two summer months is presented in the Appendix (Table A.1). The concentration of TOI for each field water sample was analyzed in duplicates under each of the 8 *treatments*. Even though the true TOI concentration in the field water samples is unknown, these runs can still help determine whether: 1) there is a statistically significant difference in concentration between the *treatments* for field water samples; and 2) if these differences align with the patterns observed for the model compounds. If both of these conditions are met, the results will further support the findings of the analysis for model compounds.

2.2.5. Instrument calibration procedure

Solutions of KI or NaI were prepared at an appropriate range of concentration and used as stock standard solutions. Instrument calibration curves were obtained by direct analysis of five to six inorganic iodide standards having a concentration range that covers expected TOI concentrations using the ICP-MS while varying Factors 2 and 3 depending on the *treatment* (Table 2.2). The data were fitted to a linear model by least squares regression with the intercept forced to zero.

2.2.6. Calculations

To calculate the concentration of TOI, the ICP-MS counts of the blank ($Blank\ count_{col\#1}$ and $Blank\ count_{col\#2}$) are subtracted from the ICP-MS counts of the sample ($Sample\ count_{col\#1}$ and $Sample\ count_{col\#2}$). The slope of the instrument standard calibrations of KI or NaI is used while taking into account the trap solution volume and the initial adsorbed volume (Eq. 2.1).

$$TOI\ (\frac{\mu g}{L}\ as\ I) = \frac{((Sample\ count_{col\#1} - Blank\ count_{col\#1}) + (Sample\ count_{col\#2} - Blank\ count_{col\#2})) \times trap\ solution\ volume}{(standard\ curve\ slope \times adsorbed\ sample\ volume)} \quad Eq. 2.1$$

Concentrations of TOI are calculated for all waters (model organic compounds, model inorganic compounds, and field water samples). For the model compounds with known initial concentrations, recovery or rejection can also be determined, while for field water samples, only the TOI concentration can be calculated, as the true value is not known. The individual recovery of the i^{th} organic compound (R^i) was calculated as the percentage of the calculated TOI concentration (Eq. 2.1) to the theoretical iodine concentration prepared (Eq. 2.2).

$$R^i\ (\%) = \frac{TOI\ (\frac{\mu g}{L}\ as\ I)}{Theoretical\ Iodine\ concentration\ (\frac{\mu g}{L}\ as\ I)} * 100 \quad Eq. 2.2$$

For organic model compounds with high recovery under a given *treatment*, random measurement error can cause recoveries to be larger than 100%. While small deviations ($\pm 5\%$) are not deemed problematic, large deviations might indicate a highly variable method that is unreliable. To account for these large deviations, a penalty approach was developed only for the eight organic model compounds to favor recoveries that were near 100% and penalize methods with recoveries that deviate greatly both

above and below 100%. This penalty is applied implicitly by conducting statistical analyses (described below) on a new quantity termed adjusted recoveries, x^i (%), equal to the negative absolute value of the difference between R^i and 100 (Eq. 2.3). The x^i values will always be less than or equal to 0, with 0 reflecting an R of 100% and increasingly negative values indicating increasingly poor recoveries. The *treatments* that produce recoveries much smaller and larger than 100% will have lower values of x^i .

$$x^i (\%) = -|100 - R^i| \quad \text{Eq. 2.3}$$

The adjusted recoveries are then standardized separately to get the mean standardized adjusted recovery y^i for each compound i , calculated as the difference between the adjusted recovery, x^i , and the mean adjusted recovery of compound i , \bar{x}^i , divided by the sample standard deviation of that compound, s_x^i , across all *treatments* and duplicates (Eq. 2.4). Larger values of y correspond to original recoveries that are closer to 100%. Standardization allows the comparison of all the compounds (upon pooling) by eliminating the systematic differences in mean recovery between the different compounds (i.e., have the mean adjusted recovery equal 0) and equalizing their standard deviations (i.e., have the standard deviation equal 1). This is particularly important when conducting statistical tests that use data pooled from all compounds.

$$y^i = (x^i - \bar{x}^i)/s_x^i \quad \text{Eq. 2.4}$$

For inorganic compounds, the individual rejection of the i^{th} compound (Rj^i) was calculated as the percentage of iodine recovery (Eq. 2.1) subtracted from 100 (Eq. 2.5).

$$Rj^i (\%) = 100 - R^i (\%) \quad \text{Eq. 2.5}$$

2.2.7. Statistical analyses

A three-way analysis of variance (ANOVA) test was performed on the eight model compounds, the two inorganic compounds, and the field water samples. More information on the ANOVA is found in the Appendix (Text, Statistical Analyses). In conjunction with the ANOVA tests, a Tukey's post hoc test was conducted on the TOI recoveries and concentrations of all water samples. The purpose of the Tukey's test is to compare and test the difference of the mean of one *treatment* with the mean of every other *treatment*. Importantly, the Tukey's test can be used to verify the *treatment* that resulted in the highest mean adjusted recovery rate of TOI across all eight organic model compounds and the highest rejection of iodine across the inorganic compounds. All statistical analyses were conducted using the *R* statistical programming language.

2.2.8. GAC breakthrough analysis

Under ideal conditions we expect 80% or more of the TOI to be retained on the first column (*Col#1*) and most of the remaining (i.e., 20% or less) to be retained on the second column (*Col #2*). Data diagnostics will be performed as a screening tool to isolate the samples that exhibited a high breakthrough from *Col#1* to *Col#2* indicating a retention ratio of $Sample\ count_{col\#1} / Sample\ count_{col\#2}$ of less than 2. This will be an indication of poor adsorption of the iodinated organics onto the two GAC columns and can act as another measure of performance for the proposed method.

2.2.9. Method detection limit

The method detection limit (MDL) procedure was conducted on IAA after statistically determining the best TOI *treatment*. Seven IAA replicates having a

concentration of 5.08 µg/L as I (between 1-5 times the expected detection level) were processed through the entire analytical method within one day. The mean concentration and the standard deviation from this mean between the seven replicates were calculated using inorganic standards after subtracting the background TOI concentration in ultra-pure water (pH = 0.99). The t-value at 99% confidence and n-1 degrees of freedom (t-value = 3.143 for seven replicates) was multiplied by the calculated standard deviation to yield a statistical estimate of the detection limit; this estimate is the MDL.

2.3 Results and Discussion

2.3.1 Method development

2.3.1.1. Model organic compounds

The recoveries (R^i) of the eight organic model compounds without any distinction between *treatments* are presented to highlight the variation in the range of values across all model compounds and to help identify any systematic differences in recovery between them (Fig. 2.1a). The overall median and mean recovery data show that some iodinated compounds, such as the 2-hydroxy-3-iodo-5-nitropyridine and the 3-iodo-4-methylbenzoic acid, are inherently better detected than others compounds, such as the two iodoacetic acids (IAA and TIAA), after averaging out variations in the method development factors. However, the recovery data show how 2-hydroxy-3-iodo-5-nitropyridine and 3-iodo-3-methylbenzoic acid can also deviate greatly above 100% recovery. This is reflected in adjusted recoveries (x^i) for these compounds, which extend well below the optimal (i.e., 0) value (Fig. 2.1b). In addition to systematic mean differences, the range of recoveries also differs across compounds. BIAA, iopromide, and

iopamidol all display a small range of recoveries, suggesting modest responses to the different *treatments*. On the other hand, compounds such as IAA and TIAA displayed a much wider range in recoveries. Upon pooling of the recoveries of all compounds, the three factors and their respective levels can be studied to better understand their influences on the recoveries (Fig. 2.1c) and the adjusted recoveries (Fig. 2.1d). The most noticeable conclusion is the improved and more stable recoveries and adjusted recoveries upon using a TMAH wash compared to using an HNO₃ wash (Factor 3). The influence of the three Factors and their statistical significance will be considered next.

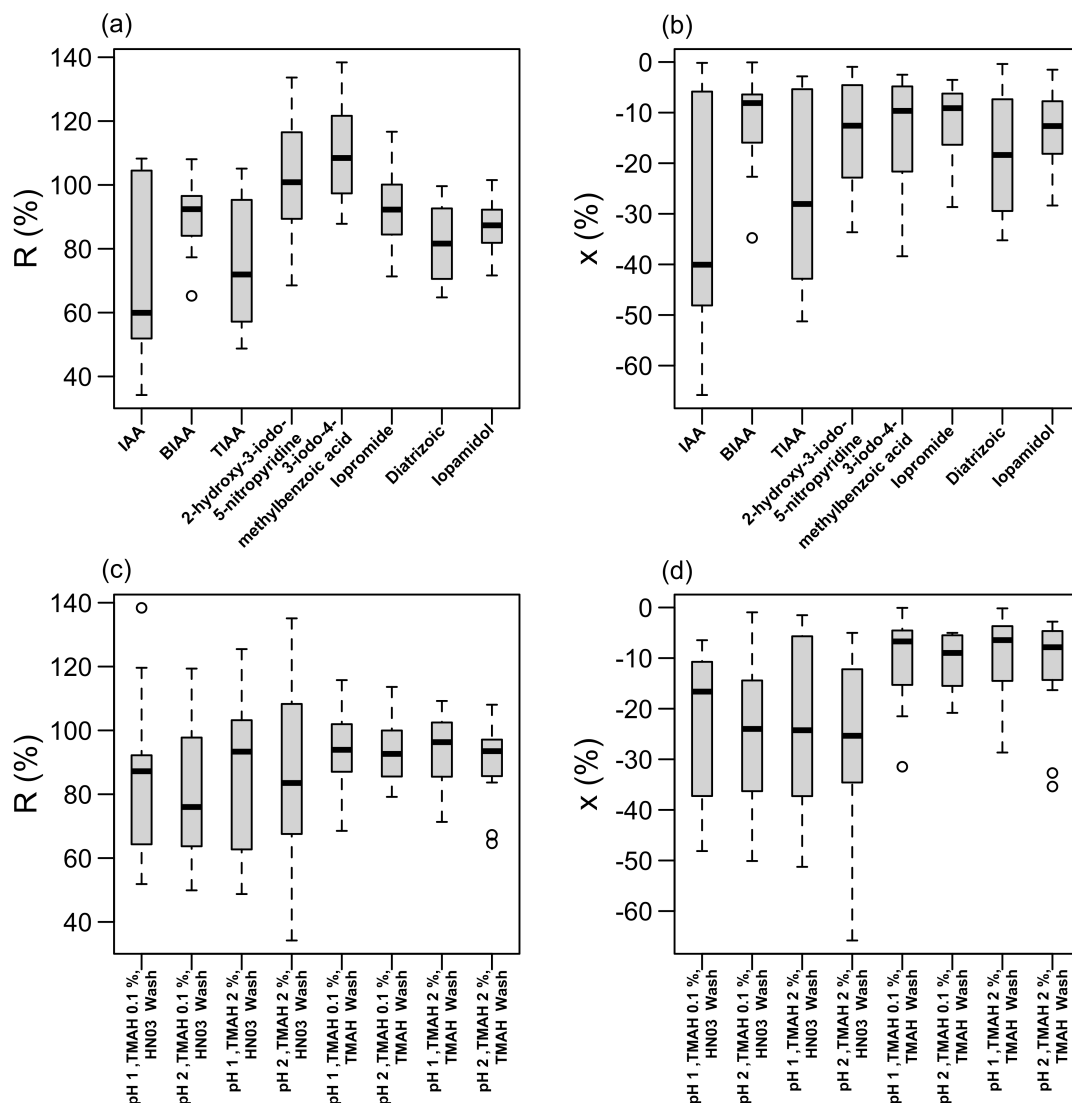


Figure 2.1: The recovery (R) of the eight model compounds ($n=16$ for each compound) regardless of the influence of factors and levels (a); the adjusted recovery (x) of the eight model compounds regardless of the influence of factors and levels (b); the recovery (R) of the eight different *treatments* upon pooling of all the compounds (c); and the adjusted recovery (x) of the eight different *treatments* upon pooling of all the compounds (d). The top and bottom of the box are 75th and 25th percentiles, respectively; the top and bottom of the whiskers are 90th and 10th percentiles, respectively; the line across the inside of the box is the median; and the circles beyond the whiskers represent outliers.

The standardized adjusted recoveries (y^i) of the eight model compounds were pooled to examine the overall effect of the three factors on recovery. A three-way interaction plot shows the independent effect of the Factors 1, 2, and 3 on the mean

standardized adjusted recovery pooled across the compounds (Figure 2.2). The pH (Factor 1) did not have a statistically significant effect on the pooled standardized adjusted recoveries of the model compounds (p-value = 0.24). This suggests that once the pH is lowered to 2, further depression in the pH does not result in better adsorption and subsequent recovery. For Factor 2, the concentration of TMAH in the trap solution (0.1% v/v versus 2% v/v) did not show a statistically significant difference in the mean standardized adjusted recoveries (p-value = 0.52). Previous studies have recommended the use of an alkaline matrix to control iodine vaporization (Reid et al., 2008; Tagami et al., 2006; Takaku et al., 1995). Our results indicate that the degree of alkalinity or pH beyond a certain point (i.e., > 0.1% v/v) in the solution itself did not affect the recovery of the model compounds. This agrees with Tagami et al. (2006) who found that varying TMAH concentrations (0 - 1.25% v/v) did not have an effect on iodine counts in the ICP-MS. The ICP-MS wash (Factor 3) was the one factor that did have a highly significant impact on the pooled, standardized adjusted recoveries of the eight model compounds. The ANOVA test verified this observation with a p-value of 2×10^{-7} . The Tukey's test was used to confirm that the TMAH (0.1% v/v) wash resulted in a significantly better mean standardized adjusted recovery than the HNO₃ (2% v/v) wash (p-value = 1×10^{-7}). The reason behind the poor performance of the HNO₃ wash could be attributed to iodine losses by volatilization in the spray chamber and nebulizer tubing walls that might occur in acidic conditions causing unstable recoveries (Al-Ammar et al., 2001; Julshamn et al., 2001; Knapp et al., 1998; Vanhoe and Van Allemeersch, 1993). It is worth noting that there were no significant interactive effects among the three factors (smallest p-value > 0.1).

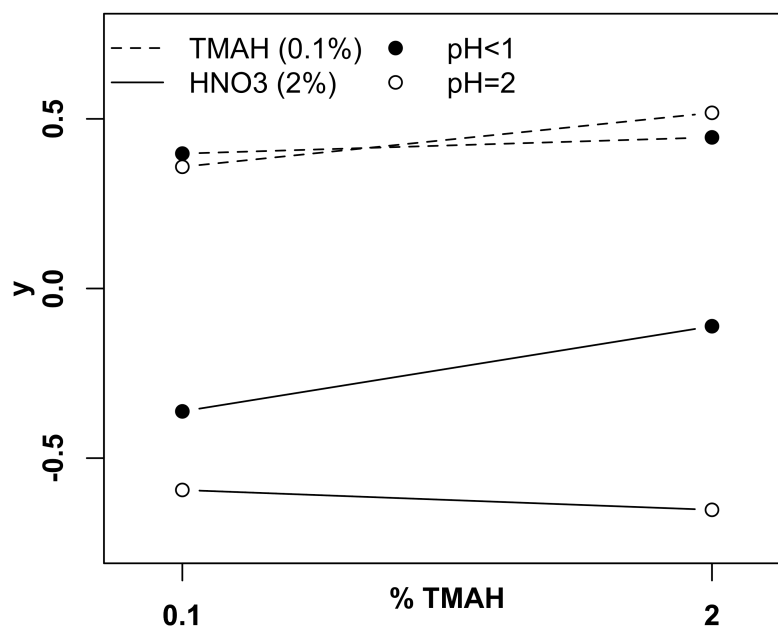


Figure 2.2: A three-way interaction plot of the means of the standardized adjusted recovery, y , of all compounds upon pooling

While the pooled data analysis allows for greater statistical power in distinguishing the effects of method development factors, we also examined the impact of the three factors and their levels on the adjusted recoveries of individual model compounds. Since two of the three factors did not show a significant impact on the adjusted recoveries when data for all compounds were pooled, additional information for method development may be available through analysis of the compound-specific adjusted recoveries. Most of the compounds showed a statistical difference of Factor 3 in the standardized adjusted recovery (y^i) (Appendix, Table A.2). Among the eight compounds, three compounds, IAA, BIAA, and iopamidol, exhibited an improved standardized adjusted recovery when the pH was less than one (Factor 1; p -value < 0.05). At a pH this low, iodinated compounds become highly protonated, which might have caused higher adsorption of these particular compounds on the activated carbon.

2.3.1.2. Inorganic compounds

Aqueous solutions of sodium iodide at two concentration levels (32 and 63 $\mu\text{g}^-/\text{L}$ as I) and separate solutions of sodium iodate at two concentration levels (14 and 69 $\mu\text{g}^-/\text{L}$ as I) were analyzed to find the *treatment* that results in the greatest rejection of these inorganic forms of I. A three-way interaction plot of the effect of the three factors on R_j^i of both I^- and IO_3^- is presented in Figure 2.3, with a distinction made between R_j^i for low and high initial concentrations of inorganic I. Upon pooling of the rejection results of both inorganic compounds at two different concentrations each, Factors 1 and 3 exhibited a significant change in rejection. At an adsorption pH of 2 (Factor 1) the recovery of inorganic iodine species was higher, presumably due to greater retention on the activated carbon (p-value < 0.01). The influence of pH was more prominent for iodide (p-value < 0.1) than for iodate (p-value = 0.39). For Factor 3, the TMAH wash resulted in significantly higher rejection of both inorganic iodine species compared to the HNO_3 wash (p-value < 0.01). This is in line with other studies that showed that an increasingly acidic ICP-MS wash causes an increase in signal intensity (Takaku et al., 1995). Therefore, for inorganic compounds, a pH of 1 prior to adsorption onto the GAC, and a TMAH wash for the ICP-MS was the best *treatment* in terms of the rejection of inorganic iodine species.

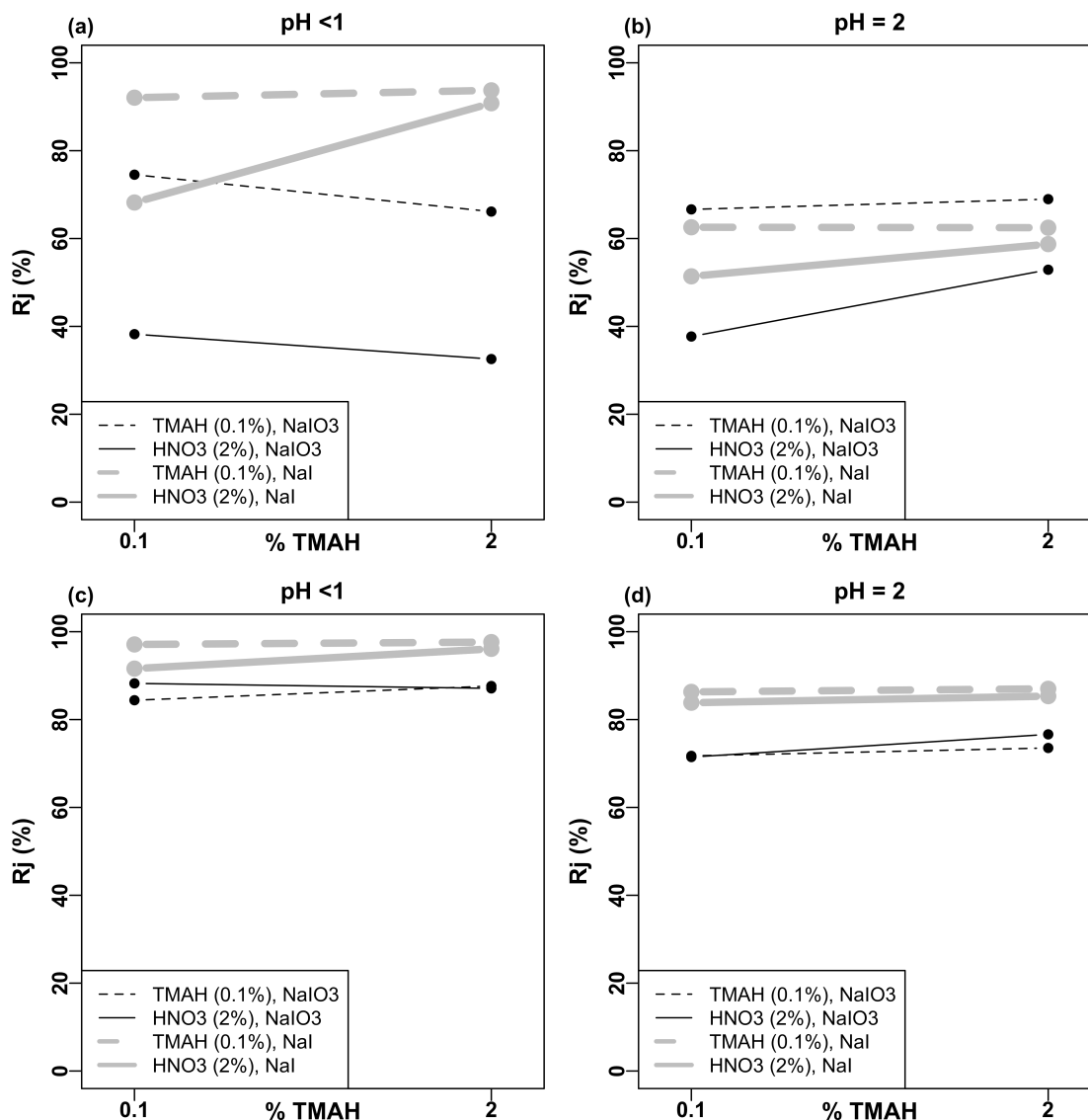


Figure 2.3: A three-way interaction plot of the three factors on the rejection of low concentrations of NaIO₃ (a) and NaI (b), and of high concentrations of NaIO₃ (c) and NaI (d)

To provide further confirmation and to examine possible interactions between inorganic iodine species, the best *treatment* was applied in triplicate to a set of prepared samples with a wider range of concentrations of iodide and iodate (5.1, 25.4 and 50.8 µg/L as I, for each separately), and to a set of equi-molar mixtures of both species (5.1, 25.4, and 50.8, µg/L as I). A 2% (v/v) TMAH in the trap solution was used since it was

more successful for some organic compounds as previously explained. For iodate, the recovered concentrations were 1.7 $\mu\text{g/L}$, 1.6 $\mu\text{g/L}$, and below detection limit for the 50.8, 25.4, and 5.1 $\mu\text{g/L}$ solutions, respectively. For iodide, the recovered concentrations were 0.9, 1.4 (duplicate only), and 0.6 $\mu\text{g/L}$ for the same concentrations, respectively. This indicates presence of a non-linear relationship between concentration of iodide or iodate and TOI rejection or recovery. This might imply that there are a small number of high energy sites within the activated carbon that strongly bind the iodide and iodate and then become quickly exhausted at high levels of inorganic iodine.

2.3.1.3. Field samples

Concentrations of TOI, rather than recoveries, are reported in the Appendix (Table A.3) for the field water samples, as the true concentrations are unknown. The TOI concentrations were comparable among the raw and treated water samples. Due to their similarities, the data from the raw and treated water samples were pooled for further analysis. Although the amount of organic iodine originally present in field samples is unknown, preventing us from calculating TOI recovery, we can study the differences in the effects of the three factors on the pooled data in a three-way interaction plot (Fig. 2.4). For Factor 1, an adsorption pH of 2 for the adsorbed samples resulted in a statistically higher concentration of TOI compared to an adsorption pH of less than 1 (p-value < 0.001). Our previous observations showed that inorganic iodide tends to get retained on the activated carbon at a pH of 2; this would explain the elevated TOI concentration in natural waters that contain iodide and iodate. To prevent this undesired outcome, a pH of less than 1 for the adsorbed samples is favored for field samples. For Factor 2, a slight increase in measured TOI was observed while using 2% (v/v) TMAH in

the trap solution compared to 0.1% (v/v) TMAH, however, this difference was not of high significance (p-value = 0.07). The presence of inorganic iodine species in the field samples may have contributed to slightly higher concentrations of TOI for the HNO₃, however, the choice of ICP-MS wash (Factor 3) was not statistically significant (p-value = 0.16). It is worth mentioning that there were no interaction effects between the three factors.

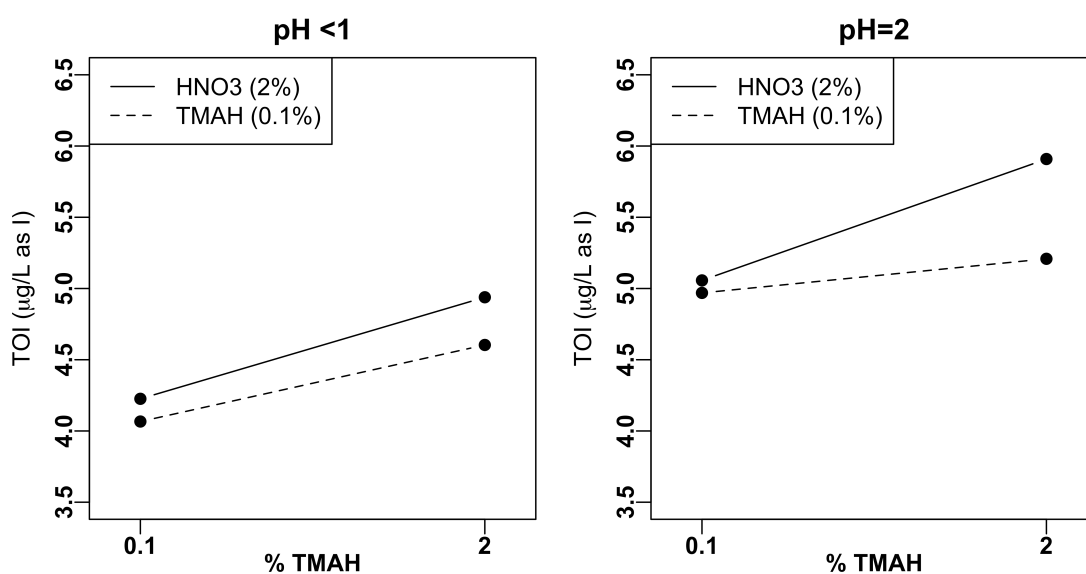


Figure 2.4: A three-way interaction plot of the three factors on the average TOI concentration in field water samples for July and August.

2.3.2 Breakthrough analysis

The retention ratio was assessed on tests with the eight organic compounds and the field samples to determine the extent of breakthrough (Fig. 2.5). The retention ratio was defined as the ratio of TOI from the first activated carbon column to the second one. A *Col#1/Col #2* ratio of 2 or below was considered to represent excessive breakthrough. For adsorption pH=2, two of the ICM compounds (diatrizoic acid and iopamidol) exhibited a high breakthrough (<2). The higher adsorption pH also resulted in poor

performance for field samples as about 30% of the samples had a high breakthrough under these conditions. This was not the case for the low pH of adsorption (< 1) where all eight organic compounds and 96% of field samples exhibited a retention ratio greater than 2.

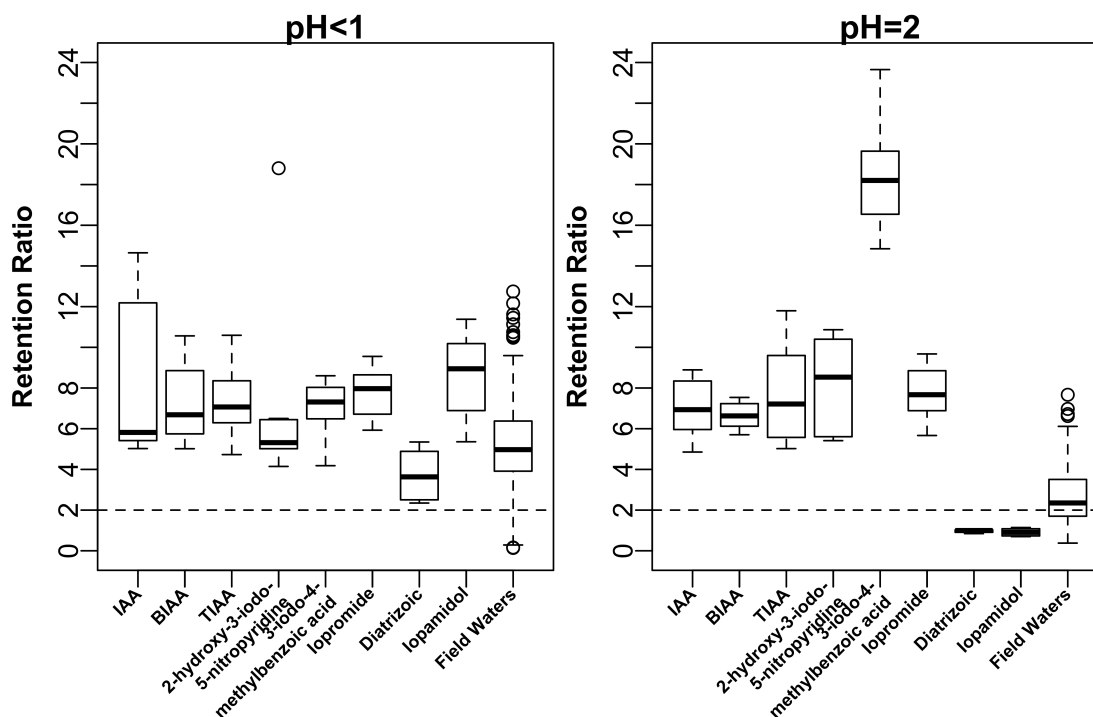


Figure 2.5: Retention ratio of the eight organic compounds and the field water samples. The cut-off point is a retention ratio of Col#1/Col #2 of 2.

2.3.3 Preferred method

The result from organic and inorganic compounds and field water samples throughout the method development procedure led us to choose the following conditions for our preferred TOI method (Appendix, Figure A.1): 1) sample pH < 1 prior to adsorption to minimize iodide retention on the activated carbon and to ensure the least breakthrough for field samples; 2) TMAH of 2% (v/v) in the trap solution prior to ICP-MS analysis since it showed slightly higher recovery of certain model iodinated organic compounds; and 3) a TMAH (0.1% v/v) wash for the ICP-MS since that wash showed

greatly improved recovery compared to the HNO_3 wash upon pooling of all the compounds, and the highest rejection of inorganic iodine species.

2.3.4. Method detection limit

The MDL for the preferred method was determined to be $0.95 \mu\text{g/L}$ as I, calculated from the analysis of seven replicates with a nominal TOI concentration of $5.08 \mu\text{g/L}$. The MDLs for TOI reported in the literature include $2 \mu\text{g/L}$ (Kristiana et al., 2009), $3.7 \mu\text{g/L}$ (Pan and Zhang, 2013), and $10.3 \mu\text{g/L}$ (Hua and Reckhow, 2006). Therefore, to the best of our knowledge, this is the lowest MDL yet reported for any TOI method. The coefficient of variation (CV), for the seven replicates used for the MDL determination was 0.059.

2.4. Implications for water treatment

The preferred method for TOI measurement entailed lowering the sample pH to less than 1 prior to adsorption onto two GAC columns, adding TMAH at 2% (v/v) to the trap solution prior to ICP-MS analysis, and using a TMAH (0.1% v/v) ICP-MS wash. This method: 1) recovered a wide range of I-DBPs, ICM compounds, as well as other iodinated organic compounds; 2) achieved the highest rejection of inorganic iodine species compared to the other tested treatments; and 3) was successfully implemented on field samples with minimum breakthrough. Using this method, ambient levels of total organic iodine can be easily measured, even in systems with low total iodine.

Given the growing interest in TOI, mainly due to the emerging toxicity literature on I-DBPs, it is important to have a sensitive, reliable, and comprehensive method for

total iodinated organics present in a raw and treated water samples. Since that was achieved, we can attempt to fully understand, characterize, and predict TOI formation in actual water treatment systems. This will be consequently covered in Chapter 3.

CHAPTER 3

OCCURRENCE OF TOTAL ORGANIC IODINE IN RAW AND CHLORAMINATED DRINKING WATER

3.1. Introduction

Disinfection by-product (DBP) formation due to the chemical disinfection of drinking water with powerful oxidants such as chlorine, chloramines, and chlorine dioxide has emerged as a prominent challenge for health agencies and water utilities. During disinfection, a myriad of organically-bound halogenated compounds form in chlorinated and chloraminated waters due to the complex and diverse nature of natural organic matter (NOM), and all cannot be feasibly identified and quantified individually. Instead, the parameter TOX (total organic halogen) has been accepted as a surrogate measure of the sum of all halogenated DBPs, or compounds that are organically-bound to chlorine, bromine, and iodine in a water sample (Li et al., 2002; Reckhow et al., 1990; Richardson, 2003). Similarly, the surrogates total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) have been adopted to account for chlorinated, brominated, and iodinated DBPs (I-DBPs) in a water sample, respectively (Oleksy-Frenzel et al., 2000; Hua and Reckhow, 2006; Hua and Reckhow, 2007; Hua et al., 2006; Kristiana et al., 2009).

In drinking water treatment plants, the use of chloramines as a final disinfectant produces considerably lower levels of regulated DBPs when compared to free chlorine (Krasner et al., 1989; Zhang et al., 2000). Consequently, to conform to the Stage 2 Disinfectant and Disinfection Byproduct Rule (USEPA, 2003), many water utilities in the US shifted from the use of free chlorine to the use of chloramines as a final disinfectant (Seidel et al., 2005; USEPA, 2012). Later studies showed that even though

chloramination produced less of the regulated DBPs, it produced more I-DBP species than free chlorine (Krasner et al., 2006; Weinberg et al., 2002; Kristiana et al., 2009; Plewa et al., 2004b; Richardson et al., 2008; Wang et al., 2016). This occurs because monochloramine does not oxidize hypiodous acid (HOI) to iodate as rapidly as free chlorine does, favoring the slow reaction of HOI with NOM to form I-DBPs (Bichsel and von Gunten, 1999; Bichsel and von Gunten, 2000). This is especially true in source waters containing high concentrations of bromide, iodide and iodate, and organic iodine (Allard et al., 2015; Bichsel and von Gunten, 2000; Criquet et al., 2012; Krasner et al., 2006; Richardson et al., 2008; Wang et al., 2016; Weinberg et al., 2002).

I-DBP formation is an emerging public health concern, especially since of the fifteen I-DBPs that have been identified in chlorinated and chloraminated water (Bichsel and von Gunten, 1999; Brass et al., 1977; Cancho et al., 2000; Chu et al., 2012; Glaze et al., 1975; Jeong et al., 2015; Krasner et al., 2006; Plewa et al., 2004b, 2008; Richardson, 2003; Weinberg, 2002), all but one have been associated with higher cyto- and geno-toxicity compared to their brominated and chlorinated analogues in mammalian cell systems (Hunter and Tugman, 1996; Jeong et al., 2015; Plewa et al., 2008, 2010; Richardson et al., 2008). Therefore, quantifying and characterizing TOI in disinfected and particularly in chloraminated waters is imperative.

Due to the recent developments in the I-DBP toxicity literature, there has been growing interest for TOI quantification in lab-treated (synthetic) and natural waters. Oleksy-Frenzel et al. (2000) used ion chromatography (IC) to differentiate between the three organically-bound halogens (TOCl, TOBr, and TOI) in the influent and effluent of a wastewater treatment plant. The TOI concentrations measured were of municipal and

hospital wastewater having high TOI concentrations (28-3060 $\mu\text{g/L}$ as I). The standard IC method has poor sensitivity for iodide (method detection limit (MDL) of about 100 $\mu\text{g/L}$ as I). Although the sensitivity can be improved by using a proper ratio of sample volume to trap solution volume (Kristiana et al., 2009), an IC run takes about 25 minutes per sample, precluding its routine analysis. A few studies have also looked at the simultaneous formation and speciation of the three TOX species (TOCl, TOBr, and TOI) in water (Hua and Reckhow, 2006; Hua and Reckhow, 2007; Hua et al., 2006; Kristiana et al., 2009; Yang et al., 2014). These preliminary studies focused primarily on method development for TOX speciation (Hua and Reckhow, 2006), formation and speciation of TOX in laboratory chlorinated (Hua et al., 2006; Yang et al., 2014) and chloraminated natural (Yang et al., 2014) and simulated drinking waters (Zhu and Zhang, 2016), and laboratory chlorinated and chloraminated NOM isolates (Kristiana et al., 2009) while adding variable amounts of iodide and bromide to the raw water. More recently, a new TOI method was developed that uses ultra-performance liquid chromatography /electrospray ionization-mass spectrometry (UPLC/ESI-MS) for iodide detection having a MDL of 3.7 $\mu\text{g/L}$ as I (Pan and Zhang, 2013). This method was used to measure TOI in 10 tap water grab samples in China (Pan and Zhang, 2013; Gong and Zhang, 2013). To date, no attempts have been made to measure and characterize TOI occurrence in drinking water treatment plants (WTPs) and their respective distribution systems without halogen augmentation. Additionally, since all of the studies were implemented either on lab-treated (synthetic) waters or on one-time grab water samples, there is a gap in our understanding of how TOI forms in real-world engineered systems over extended periods of time.

To the authors' knowledge, this work presents the first full-scale study of several drinking WTPs in the US that explores the occurrence of TOI in raw and chloraminated drinking water without the addition of iodine or bromine sources. A recently developed, highly sensitive method (MDL 0.95 $\mu\text{g/L}$ as I) was used for TOI determination (Sayess and Reckhow, 2017). In the context of the aforementioned knowledge gaps, we characterize the magnitude and seasonal behavior of TOI and the contribution of TOI to total iodine (TI) concentrations in three WTPs. We use that information to pose the following three hypotheses: 1) TOI concentration significantly increases upon treatment; 2) the organic fraction of TI changes between raw and treated water; and 3) TOI occurrence is influenced by commonly measured physical and chemical properties in a treated water sample. The ultimate goal of this line of research is to better understand and predict the occurrence of TOI in treated water to enable water utilities to better assess the presence of iodinated organics in the absence of advanced analytical techniques.

3.2. Materials and Methods

3.2.1. Instrumentation

The instrumentation for TOI analysis includes adsorption and combustion units and an off-line inductively coupled plasma – mass spectrometer (ICP-MS). The adsorption systems used was either an EFU 1700 Filtration Unit (Euroglas BV, Delft, The Netherlands) or an XPREP-A6 (Trace Elemental Instruments, Delft, The Netherlands), both equipped with pressurized sample reservoirs and granular activated carbon (GAC) adsorption columns (CPI International, Santa Rosa, CA). The ECS 1200 combustion system (Euroglas BV, Delft, The Netherlands) includes a combustion glass tube, a boat sampler, a motor-driven boat sampler, a furnace, sulfuric acid scrubbers, a

gas bubbler/diffuser and trap, and an oxygen gas supply (99.99% high-purity grade). The ICP-MS (Perkin Elmer Elan 9000) was used for measuring iodide concentration in the trap solution for TOI measurement, as well as to measure the TI and total bromine (TBr). Argon gas (99.99% high-purity grade) was used as the carrier and reaction gas. All other analyses were done according to the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1998).

3.2.2. Chemicals and reagents

Ultra-pure water was obtained by filtering de-ionized water with a resistivity greater than 18.3 MΩ.cm (Billerica, MA) and used in preparing procedural calibration standard solutions, laboratory reagent blanks, model compound solutions, and the ICP-MS wash solutions. Potassium iodide (KI; Certified ACS grade), sodium iodide (NaI; Certified ACS grade), sodium iodate (NaIO₃; Certified ACS grade), potassium bromide (KBr; Certified ACS grade), and nitric acid (70%, Trace Metal grade) were obtained from Fisher Scientific. Tetramethylammonium hydroxide (TMAH, 25% W/W aqueous solution, Electronic Grade 99.999%) was obtained from Alfa Aesar.

3.2.3. Field samples

All raw and treated water samples were collected from the same upstream and downstream locations throughout the sampling period in three WTPs (A, B, and C) and their distribution systems in the United States Environmental Protection Agency (USEPA) Ecoregion 3 (Figure 3.1). Ecosystems within the same ecoregions generally have similar quality, type, and quantity of environmental resources (USEPA, 2016). The “A” sampling train originates from a large river (basin of about 14,000 square miles)

while “B” and “C” originate from a small river (basin of about 2,000 square miles). Three raw waters (A-Raw, B-Raw, C-Raw) were collected from the intakes of the two rivers feeding into the three WTPs. At each of the WTPs, a water sample was collected from the point of entry into the distribution system (A-POE, B-POE, and C-POE), in addition to two points in the distribution system (A-DS1 and A-DS2, B-DS1 and B-DS2, and C-DS1 and C-DS2). In all of the discussion to follow, we distinguish the treatment trains A, B, and C as “sites” and the sampling locations along a treatment chain (Raw, POE, DS1, DS2) as “locations.” In total, there were 12 samples (three raw and nine treated) collected for 3 months in 2014 (January, June, and July), and 12 consecutive months from May of 2015 to April of 2016. Samples were collected within 1-2 days between the raw and treated waters. Although this indicates that the same plug of water is not followed throughout the distribution sampling train, we assume small changes in water quality within a range of a couple of days after confirming that the streamflow and precipitation data on the days of collection were similar.

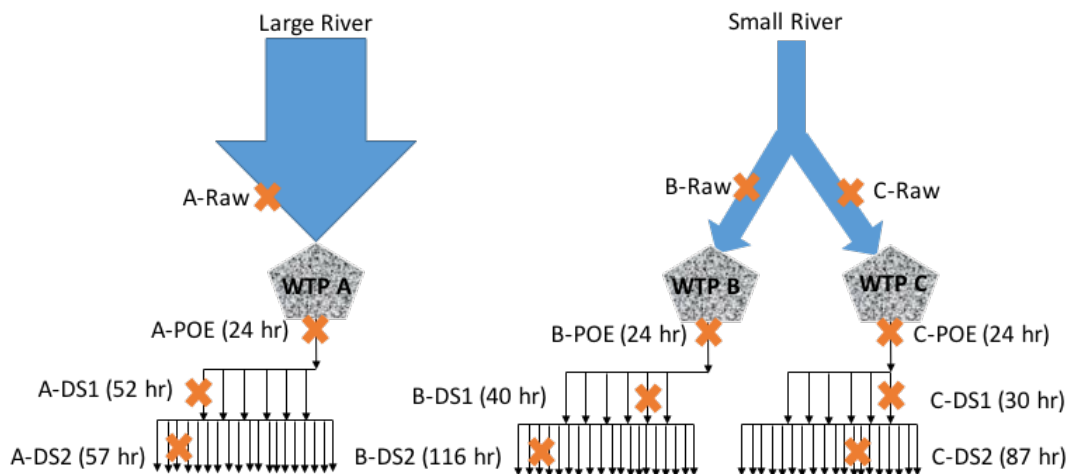


Figure 3.1: A schematic of the 12 sampling locations with respect to the three sites (not to scale)

The treatment process in the three WTPs involves pretreatment, coagulation, settling, filtration and disinfection. Disinfection of the water is applied as chlorine at pretreatment and during coagulation, and after filtration as chloramine. More detailed information on the chlorination and chloramination for the three WTPs is presented in Table 3.1. Ascorbic acid was added to each of the collected samples as a quenching agent prior to being transported overnight using ice coolers to the University of Massachusetts, Amherst. The range of values of some of the physical and chemical characteristics collected over the 15 months are presented in the Appendix (Table A.4).

Table 3.1: Information on chlorination and chloramination of the three intensively studied WTPs

WTP	Primary chlorination dose (mg/L)	Ferric chloride dose (mg/L)	Secondary chlorination dose (mg/L)	Contact time (min)	Ammonia dose (mg/L as N)	Cl ₂ /N
A	2.6	30.6	2.2	65	0.64	3.7
B	3.1	43	2.04	49	0.7	4.0
C	1.8-4.2	43-53	2-3.5	66	0.7	Not specified

Grab samples from four other sites (D, E, F, and G) located in four other USEPA ecoregions (Regions 1, 4, 5, and 9) in the continental US were also collected during the summer months of 2015 and 2016 for comparative purposes (Table 3.2). Collecting samples from different USEPA ecoregions will allow us to study the impact of the inherent characteristics that are unique to each ecoregion on TOI concentrations. For each of the four sites, samples were collected from raw (surface) and treated (POE) waters, except for site D where the samples were collected from raw water and at a point in the distribution system (DS). WTPs E and G use chloramine as the final disinfectant prior to release into the distribution system, while WTPs D and F use only free chlorine. Water

from site G also underwent pre-ozonation in addition to free chlorine and ammonia addition.

Table 3.2: Information on grab sample collection from the four other WTPs

WTP	EPA Ecoregion	Samples	Treatment	Final disinfectant
D	1	Raw and DS	Coagulation, sedimentation, sand filtration	Free chlorine
E	4	Raw and POE	Pre-free chlorine, coagulation, sedimentation, sand filtration	Chloramine
F	5	Raw and POE	Coagulation, sedimentation, sand filtration	Free chlorine
G	9	Raw and POE	Ozone, Pre-free chlorine, coagulation, sedimentation, sand filtration	Chloramine

3.2.4. Analyses

The detailed method for TOI analysis is described in Chapter 2 and in Sayess and Reckhow (2017). In short, samples were first acidified to a pH of less than 1. Fifty mL of the acidified samples was then adsorbed onto a module of two granular activated carbon (GAC) columns at a flow rate of 3 mL/min. The two GAC columns were then sequentially placed in a ceramic boat that is introduced into a 1000 °C oven to be combusted in the presence of oxygen gas for 10 minutes. Upon combustion, the organic iodine is released as HI in the gas phase that gets carried through sulfuric acid scrubbers and into a custom-made absorber cell that contains about 15 mL of ultra-pure water (the trap solution). At the end of the trapping cycle, the trap solution was adjusted to 20 mL by rinsing out the walls of the absorber cell. TMAH (2% v/v) was then added to the 20 mL trap solution leading to a trap solution pH of about 12. The trap solution was then analyzed using the ICP-MS. The ICP-MS wash solution used was TMAH at 0.1% v/v. To account for any background iodine, an ultra-pure Milli-Q travel blank was analyzed along with each set of tested samples. Solutions of KI or NaI were prepared at an appropriate range of concentration and used as stock standard solutions. Instrument calibration curves

were obtained by direct analysis of five to six inorganic iodide standards having a concentration range that covers the expected TOI concentrations using the ICP-MS. The data were fitted to a linear model by least squares regression with the intercept forced to zero. This TOI method has a MDL of 0.95 µg/L and a coefficient of variation of 5.9%.

The ICP-MS instrument was also used to determine TI and total bromine (TBr). Samples being analyzed for TI and TBr were first adjusted by adding 0.1% v/v of TMAH to the samples prior to their direct injection into the ICP-MS. Similar to TOI analysis, procedural calibration standards of the inorganic halogens were used to calculate the concentration of TI and TBr after accounting for the concentrations of the iodide and the bromide in ultra-pure Milli-Q blanks. The MDL for TI and TBr using the ICP-MS is 0.19 and 0.98 µg/L as I and as Br, respectively.

The different physical and chemical parameters measured and their associated measurement methods are presented in Table 3.3. Specific UV₂₅₄ absorbance (SUVA₂₅₄) was calculated as follows (Eq. 3.1):

$$SUVA \left(\frac{L}{mg-m} \right) = (UV_{254} * 100) / DOC \quad \text{Eq. 3.1}$$

Table 3.3: Physical and chemical parameters measured and their associated methodologies

Parameter	Method or Instrument
Total organic carbon (TOC) and dissolved organic carbon (DOC)	Standard Method 5310B (APHA et al., 1995)
pH	Standard Method 4500-H+.B. (APHA et al., 1995)
Ammonia-nitrogen (NH ₃ -N)	Standard Method 4500-NH ₃ .D. (APHA et al., 1995)
Nitrate-nitrogen (NO ₃ -N)	EPA 300.0 (EPA, 1993)
Temperature (T)	Calibrated thermometer
UV ₂₅₄	Standard Method 5910B (APHA et al., 1995)
Total nitrogen (TN)	Shimadzu TOCV
Conductivity	Standard Method 2520B (APHA et al., 1995)
Combined chlorine residual (TCl ₂)	Standard Method 4500-Cl G. (APHA et al., 1995)
Chloride	EPA 300.0 (EPA 1993)

3.2.5. Calculations

To calculate the concentration of TOI, the ICP-MS counts of the travel blank ($Blank\ count_{col\#1}$ and $Blank\ count_{col\#2}$) are subtracted from the ICP-MS counts of the sample ($Sample\ count_{col\#1}$ and $Sample\ count_{col\#2}$). The slope of the instrument standard calibrations of KI or NaI is used while taking into account the trap solution volume and the initial adsorbed volume (Eq. 3.2).

$$TOI\left(\frac{\mu g}{L}\text{ as I}\right) = \frac{((Sample\ count_{col\#1} - Blank\ count_{col\#1}) + (Sample\ count_{col\#2} - Blank\ count_{col\#2})) \times trap\ solution\ volume}{(standard\ curve\ slope \times adsorbed\ sample\ volume)}$$

Eq. 3.2.

3.2.6. Data screening

3.2.6.1. GAC breakthrough analysis

Data diagnostics were performed as a screening tool to isolate the samples that exhibited a high breakthrough from the first GAC column to the second ($Col\#1$ and $Col\#2$), indicated by a low retention ratio of $Sample\ count_{col\#1} / Sample\ count_{col\#2}$. This will be an indication of poor adsorption of the iodinated organics onto the two GAC columns. We have excluded any samples with a retention ratio less than 2. This resulted in the exclusion of 12% of the samples.

3.2.6.2. Outliers

An additional step was taken to exclude outliers in the data. For the purpose of this study, outliers are identified based on whether the TI value is less than the TOI value, allowing for some deviation based on TOI measurement error (TI measurement error is comparatively small and ignored here). To quantify measurement error for TOI, the

coefficient of variation (CV) was first calculated from a previous data set of duplicates of iodinated model organic compounds (Sayess and Reckhow, 2017) as well as duplicates from two months of the current TOI data set. Here, one CV value is calculated for each duplicate. The mean CV across duplicates was then calculated separately for both datasets: 0.12 and 0.10 for the real water samples and model compounds, respectively. Their average CV value (0.118) was used to calculate an estimate of the standard deviation (SD) of each TOI value in the current dataset. Finally, samples were excluded if the lower bound of a 95% confidence bound ($\text{TOI} - 1.96 \times \text{SD}$) was larger than the associated TI measurement. This resulted in the exclusion of 2% of the samples.

3.2.7. Data analysis

3.2.7.1. Occurrence

To characterize the occurrence of TOI in chloraminated samples, several types of observations will be presented, including the order of magnitude of TOI in raw and treated waters, as well as its seasonal variability. These data will be presented along with TI for the same samples. For comparison purposes, we will present the concentrations of TOI from raw and treated grab samples from four other sites representing four other ecoregions in the continental US. We also examine the ratio of TOI to TI in order to develop a better estimate of the distribution of inorganic iodine (iodide and iodate species) and organic iodine in fresh water sources and their respective treated waters in the Northeast US. For all comparisons of TOI and TI concentrations across sites or months, an analysis of variance (ANOVA) was conducted to establish the statistical

significance for mean differences, and a Tukey's post hoc test is used to verify the direction of those differences.

3.2.7.2. Influence of treatment

Since sampling in a given month did not follow the exact same plug of water from raw through to the treated samples, we pooled the results from all of the 15 months into a single statistical testing framework. This will allow us to study whether, on average, TOI significantly changes upon treatment. One approach to test this hypothesis would include multiple t-tests between the raw and different downstream (POE, DS1, DS2) samples, but this multiple-comparison approach would augment Type I error (i.e., incorrectly rejecting the null hypothesis that there is no change in TOI concentration between the raw and treated water). Therefore, a one-way ANOVA is conducted to avoid multiple comparisons. This permits us to test the difference in mean TOI concentration depending on the location in the distribution system (Raw, POE, DS1, and DS2). Separate ANOVA tests are conducted for sites A, B, or C due to differences in the mean and variance of TOI across the sites. The particular locations within the distribution system will be the four levels in the ANOVA. In the ANOVA, the general term y_{ij} is used to represent the j^{th} observation on the i^{th} location ($i= 1, 2, 3$, and 4 level) representing the four locations within a sampling train (Eq. 3.3).

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad \text{Eq. 3.3}$$

Here, μ is the overall mean of the observations, τ_i represents the i^{th} location effect, and ε_{ij} is the random noise/error component present in the j^{th} observation on the i^{th} location. If there is a statistical difference in the average TOI between any of the four

locations, this would indicate that the average TOI concentration in the raw water is different from at least one of the locations downstream. Then, in conjunction with the ANOVA tests, a Tukey's post hoc test was conducted to compare and test the difference of the mean of one location with the mean of every other location to determine the direction of differences in TOI between raw and treated waters.

In addition to TOI, we perform a similar analysis on TI and on the ratio of TOI to TI in the raw and treated waters. This will help determine whether the total amount of iodine or the speciation of iodine between inorganic and organic forms changes significantly under treatment.

3.2.7.3. Relationship with commonly measured parameters

Another objective of this work is to explore the existence of a relation between TOI and some of the physical and chemical parameters that are commonly measured during routine water quality analyses. Our focus here is placed on the treated water samples since we are interested in the quality of the water that reaches the consumer. Therefore, for the purpose of this analysis, the raw water samples were not included. To test this hypothesis, we employ a multiple linear regression framework to relate TOI concentrations to a suite of physical and chemical properties. A step by step procedure is summarized here. The parameters (covariates) in the regression are shown in Table 3.4, along with the correlation coefficients between the various parameters. It is important to point out that several of the parameters are already highly correlated with one another, such as chloride and conductivity, and dissolved organic carbon (DOC) and total organic carbon (TOC). Collinearity among predictor variables increases the standard error on regression parameter estimates and reduces the ability to distinguish between significant

and insignificant predictors. A simple, yet effective approach to reduce collinearity in a regression framework is simply retaining some predictors over others if the correlation coefficient between the covariates is greater than some threshold value (Dorman, 2013). For interpretability, we adopt this simple approach, setting the threshold for Pearson r to 0.6 and retaining predictors that are most likely to explain TOI variability from a mechanistic perspective (highlighted in bold in Table 3.4). The retained variables for the multiple linear regression framework were TI, temperature (T), pH, combined residual chlorine (TCI_2), DOC, ammonia-nitrogen ($\text{NH}_3\text{-N}$), specific UV_{254} absorbance (SUVA_{254}), and chloride.

Table 3.4: The correlation coefficients of the measured parameters with the ones in bold indicating the ones chosen for the regression analysis. Correlation coefficients greater than or equal to 0.2 are significant at the 0.01 level.

	TI	T	pH	Conductivity	TCI_2	TOC	DOC	UV_{254}	$\text{NH}_3\text{-N}$	$\text{NO}_3\text{-N}$	SUVA_{254}	Chloride	TBr	TN
TI														
T	0.6													
pH	0.2	0.1												
Conductivity	0.5	-0.1	0.4											
TCI_2	-0.3	-0.2	0.0	-0.3										
TOC	0.6	0.4	0.3	0.5	-0.4									
DOC	0.5	0.4	0.3	0.5	-0.3	0.9								
UV_{254}	0.6	0.6	0.2	0.4	0.0	0.7	0.7							
$\text{NH}_3\text{-N}$	0.3	0.3	0.2	0.3	-0.5	0.4	0.3	0.4						
$\text{NO}_3\text{-N}$	0.4	-0.3	0.3	0.9	-0.3	0.4	0.4	0.3	0.4					
SUVA_{254}	-0.1	0.1	-0.1	-0.2	0.4	-0.5	-0.6	0.2	0.0	-0.3				
Chloride	0.1	-0.4	0.2	0.6	-0.2	0.2	0.3	0.1	0.2	0.6	-0.3			
TBr	0.6	0.3	0.3	0.5	-0.4	0.6	0.4	0.4	0.2	0.4	-0.2	0.2		
TN	0.4	0.2	0.3	0.8	-0.2	0.7	0.6	0.6	0.5	0.7	-0.2	0.7	0.5	

The formulation of the multivariate regression is complicated by the measured TOI values that were below the MDL of $0.95 \mu\text{g/L}$. While numerical values are available for these observations, the MDL renders them indistinguishable from background noise.

Therefore, assigning the numerical values for these observations equal weight to the other observations in a standard regression will lead to inconsistent regression coefficient estimates (Amemiya, 1973). A Tobit model, a type of multivariate censored regression model, is used to correct for this and to assign the appropriate level of precision to these observations in the regression. Briefly, the Tobit model operates using maximum likelihood estimation instead of least squares. In the likelihood function, the probability density function (pdf) is evaluated for the residuals associated with the data above the MDL, since those residuals can be estimated precisely. For the data below the MDL, the residuals cannot be estimated precisely because the original data values are not known. For these residuals, we evaluate the cumulative distribution function (cdf) with integration limits determined by the MDL. In the likelihood function, the pdf values will naturally have more weight than the cdf values, giving more emphasis to the data we know more precisely. Similar to standard regression models, p-values can be used to test the significance of each covariate. The Tobit model was fit using the *censReg* library (Henningsen, 2011) in the *R* statistical programming language. After determining the statistically significant parameters on TOI concentration, the Tobit regression was refit using only those parameters. The R^2 was calculated by assigning a value of half the MDL (0.475 $\mu\text{g/L}$) for values that were below the MDL (0.95 $\mu\text{g/L}$). The significance (p-value) of the final Tobit regression model was determined using a likelihood ratio test against a null model without any covariates.

3.3. Results and Discussion

3.3.1. Occurrence

The TOI and TI concentrations at sites A, B, and C are examined first by pooling across months and sampling locations (Figure 3.2). The original, un-pooled TI and TOI data across sites, sampling locations, and months are shown in the Appendix (Figure A.2). For TI, the lower end of the concentration range was around 3 $\mu\text{g/L}$ in the raw and treated water at all sites. The median TI concentration was 5 $\mu\text{g/L}$ for site A, and 11 $\mu\text{g/L}$ for sites B and C. The higher end of the concentration range was 11.5, 23.1, and 18.9 $\mu\text{g/L}$ for sites A, B, and C, respectively. Many studies have reported on TI concentrations in fresh water sources (Table 3.5). Takaku et al. (1995) reported on rivers in Kanto region, Japan, having TI values ranging between 0.65 and 35.9 $\mu\text{g/L}$. In the United States, TI values in some rivers ranged between 0.06 $\mu\text{g/L}$ to 26.9 $\mu\text{g/L}$ (Moran et al., 1997; Moran et al., 1999; Moran et al., 2002; Oktay et al., 2000; Oktay et al., 2001). The TI concentrations in Lake Constance, Germany, and its tributaries ranged between 0.66 and 10 $\mu\text{g/L}$, with the exception of the Steinach tributary which is known to have an input of iodinated X-ray contrast media compounds (Gilfedder et al., 2010). For treated waters, Gong and Zhang (2013) reported on TI concentrations in four chlorinated grab samples in four WTPs in China collected in August 2013 to be between 6.5 and 12.9 $\mu\text{g/L}$. In comparison, results from our grab samples for WTPs E, F, and G (the TI concentration for WTP D was not available) were between 4.9 and 14.26 $\mu\text{g/L}$ in the raw water and between 3.5 and 8.3 $\mu\text{g/L}$ in the treated water. Overall, the TI results obtained in our study are in line with those reported in the literature.

Table 3.5: Comparison between the TI concentration in the literature and this study

	TI concentration ($\mu\text{g/L}$) in raw waters	TI concentration ($\mu\text{g/L}$) in treated waters
Takaku et al., 1995	0.65 – 35.9	NA
Gilfedder et al., 2010	0.6 – 10	NA
Gong and Zhang, 2013	NA	6.5-12.9
WTPs A, B, and C (this study) ^a	3.7 – 19.2	2.5 – 23.1
WTPs E, F, and G (this study) ^b	4.9 – 14.2	3.5 – 8.3

^a The results of the extensive sampling over the 15-month period

^b The results of the grab samples

When examining TOI at site A, about half of the TOI values were below the MDL while the higher end of the range was about 4 $\mu\text{g/L}$ for the raw and treated waters. The maximum TOI values observed for site B were 11.75 $\mu\text{g/L}$ for the raw water and 17.7 $\mu\text{g/L}$ for the treated water, with a median of about 3 $\mu\text{g/L}$ for both. For site C, maximum TOI concentrations in the pooled raw and treated waters reached about 10 $\mu\text{g/L}$, also with a median around 3 $\mu\text{g/L}$. By pooling across months and sampling locations, the results showed that site A had significantly lower TOI concentrations than sites B and C (p-value < 0.005). This relationship was also evident when assigning a value of half the MDL (0.475 $\mu\text{g/L}$) for the samples that had a TOI concentration below the MDL. The lower TOI concentration in site A could be seen as directly related to the amount of TI in the raw and treated water, which was significantly lower in site A as well (p-value < 0.001). The pooled TOI concentrations across the months and sampling locations did not show any statistical differences between sites B and C (p-value > 0.05). Considering that the same source water feeds into WTPs B and C that operate in a similar manner (Table 3.1), these results are not surprising.

For comparison, the TOI concentration in the raw and treated water grab samples collected from four sites (D, E, F, and G) are presented in Table 3.6. The TOI concentration in the raw and treated waters from the four other sites ranged between 1 and 4 $\mu\text{g/L}$. The order of magnitude of TOI presented for these sites and for the bulk of the samples collected for sites A, B, and C are in line with results of raw (Gilfedder et al., 2010) and chlorinated grab tap water samples (Pan and Zhang, 2013; Gong and Zhang, 2013) in the literature. The TOI concentration in Lake Constance, Germany, and its tributaries was calculated from TI and total inorganic iodine (TII) to be generally below 5 $\mu\text{g/L}$, with the exception of the Steinach tributary which had a range of 2 to 68 $\mu\text{g/L}$ (Gilfedder et al., 2010). For treated samples, TOI concentrations, measured using an UPLC-ESI/MS, were as low as 1.3 $\mu\text{g/L}$ but reached up to 16.4 $\mu\text{g/L}$ in chlorinated grab water samples (Pan and Zhang, 2013; Gong and Zhang, 2013; Pan et al., 2016). The skewness in TOI concentrations in treated samples in the literature is also observed at sites B and C, with a few TOI values substantially larger than the majority of measurements. Repeated sampling at these sites B and C suggest that limited grab samples may miss important spikes in TOI concentrations.

The ratio of TOI/TI at sites A, B, and C are examined next by pooling across months and sampling locations (Figure 3.2). The average ratios for sites A, B and C were roughly the same, with the mean ratio for site A (0.30) slightly lower than those for Sites B and C (0.35). Not only were the three sites similar in their means, but all three sites displayed highly variable ratios across the months and sampling locations. The inter-quartile range for ratios varied between 0.04 and 0.50 for site A and between 0.15 and 0.56 for sites B and C. On many occasions at all sites, spikes in TOI values were not

accompanied by spikes in TI and vice versa, leading to the high variability in ratio values (Appendix, Figure A.2). Results from WTPs E, F, and G (TI values for WTP D were not available) showed that the organic fraction ranged between 0.22 and 0.41. The results from all the WTPs are in line with the literature. For example, Heumann et al. (1998) reported organic iodine fractions of 0.23, 0.38, and 0.38 of TI in Rivers Nile, Regen, and Danube, respectively, using reverse phase chromatography/inductively coupled plasma-isotope dilution mass spectroscopy (RPC/ICP-IDMS). However, the authors reported that this method may have missed high molecular weight or volatile organic species which would have increased the organic fraction of TI. Shwehr and Santschi (2003) reported that the organic iodine (calculated as the difference between TI and TII) fraction in fresh surface water from the Trinity River, Texas, ranged between 0.07 to 0.64 of TI, with an average of 0.37 and a median of 0.42. Others reported an even bigger TOI fraction, reaching up to 1 in several natural fresh waters using an isotope dilution mass spectrometry that had a similar problem as RPC/ICP-IDMS (Reifenhäuser & Heumann, 1990). Overall, the high variability in the organic fraction observed here is not uncommon.

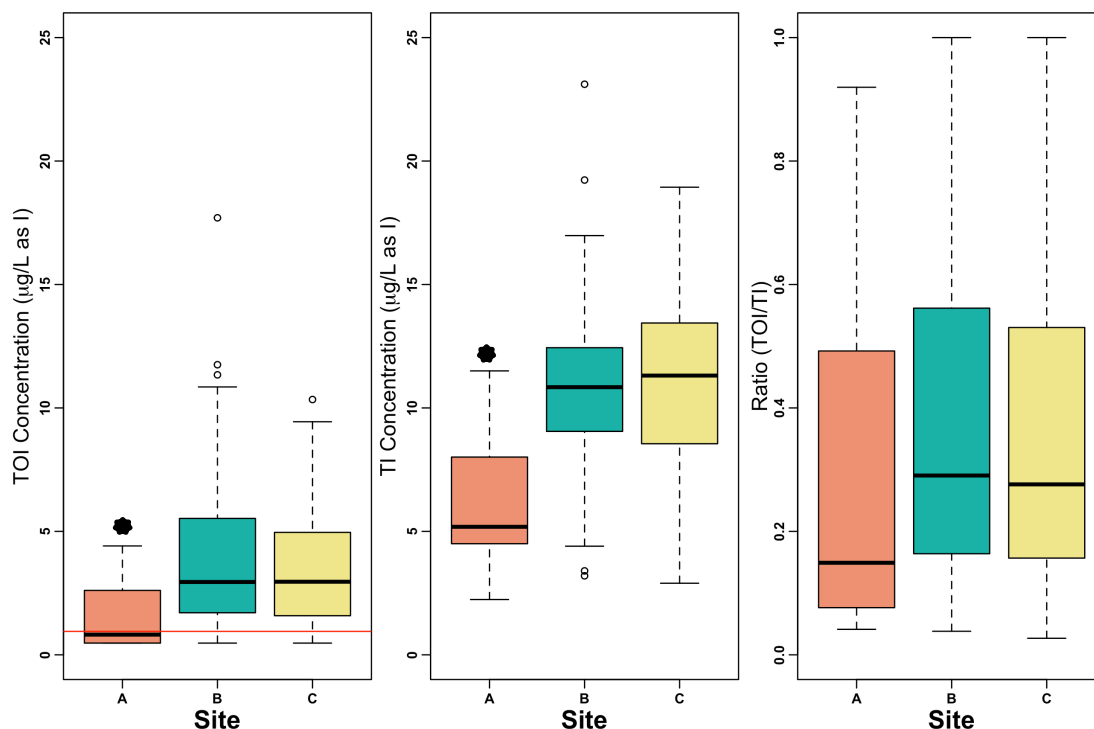


Figure 3.2: The concentration of TOI (left), TI (center), and the ratio of TOI to TI (right) at sites A, B, and C pooled across the months and sampling locations. (left) The horizontal red line represents the TOI MDL of 0.95 µg/L. TOI values that were below the MDL (n=35) were assumed as half the MDL (0.475 µg/L). Ratios of TOI/TI that were slightly greater than 100% (see Section 3.2.6.2) were forced to 100% for clarity. The black star indicates significant difference between the sites (p-value < 0.05).

Table 3.6: Concentration of TOI and TI and the ratio of TOI/TI in the grab raw and treated samples in the four other WTPs.

WTP	EPA Ecoregion	TOI (µg/L)		TI (µg/L)		TOI/TI	
		Raw	POE or DS	Raw	POE or DS	Raw	POE or DS
D	1	3.97	3.06	NA	NA	NA	NA
E	4	3.85	2.33	14.26	8.33	0.27	0.28
F	5	1.09	1.27	4.95	3.48	0.22	0.36
G	9	2.62	2.01	6.41	7.28	0.41	0.27

The results of TOI, TI, and ratio of TOI/TI for the fifteen months pooled across all sites and sampling locations are presented in Figure 3.3. We assume here that any systematic mean differences between sites A, B, and C or sampling locations are consistent across months. For TI, the primary seasonal pattern is characterized by higher

concentrations in the spring and summer months (April – September) compared to concentrations in the fall and winter months (October – March) ($p\text{-value} < 0.001$). This pattern, however, is not observed for TOI where the seasonal pattern is somewhat less clear. There is substantial inter-monthly variability in TOI measurements, in which high or low values of TOI cluster for 2-3 months at a time (July to August 2016, October to December 2016). Gilfedder et al. (2009) reported the highest formation of soluble organic iodine (calculated, not measured) in the humic rich Mummelsee Lake ($\text{DOC} \sim 7 \text{ mg/L}$), Germany, between July and November. Apart from September, this pattern is similar to what has been observed in this study. Therefore, we conclude that there may be a cyclic pattern in TOI variability similar to that reported in Gilfedder et al. (2009), with peak values between the late summer and early winter, but additional years of data are necessary to confirm this pattern. The TOI/TI ratios become similarly elevated in the late summer and early winter, compared to the other seasons. The cyclic variability in TOI seems to highly influences the TOI/TI ratios as this ratio mostly follows the observed occurrence trends of TOI.

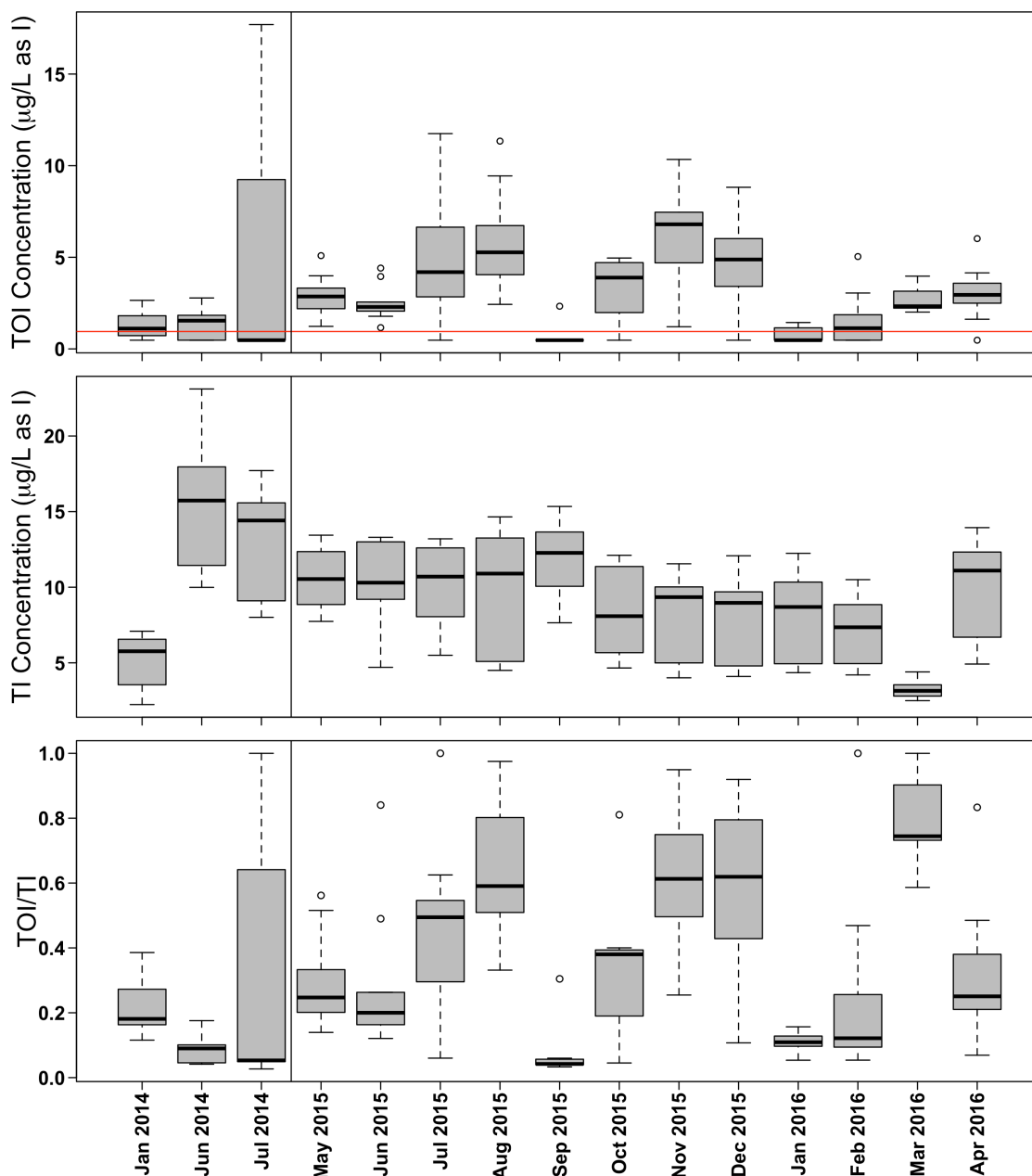


Figure 3.3: The concentration of TOI (top), the concentration of TI (middle), and the ratio of TOI to TI (bottom) for every month pooled across all sites and locations. The horizontal red line represents the TOI MDL of 0.95 µg/L. TOI values that were below the MDL (n=35) were assumed as half the MDL (0.475 µg/L). Ratios of TOI/TI that were slightly greater than 100% (see Section 3.2.6.2) were forced to 100% for clarity. The vertical black line separates the three 2014 sampling dates from the other twelve.

3.3.2. Influence of treatment

It is well established in the literature that I-DBPs form in chloraminated water. So far, the formation of six iodinated trihalomethanes (I-THMs) (Hansson et al., 1987; Bichsel and von Gunten, 2000; Krasner et al., 2006), five iodoacids (Krasner et al., 2006; Plewa et al., 2004; Wei et al., 2013), two iodoacetaldehydes (Krasner et al., 2006), and two iodoacetamides (Chu et al., 2012; Plewa et al., 2008) have been identified in chloraminated water. The hypothesis examined here was that the TOI in the raw water increases upon treatment due to the increase in I-DBP formation. To test this hypothesis, the influence of treatment on TOI concentration was explored at each of the sites (Figure 3.4). Somewhat surprisingly, we only detect a significant change between TOI in the raw water and the treated water at site A (p-value = 0.0467). Sites B and C show no significant differences (p-value > 0.05). Moreover, the Tukey's test showed that that change in TOI at site A was due to higher TOI concentration in A-Raw compared to A-DS1 (p-value = 0.033), which ran counter to our expectation of higher TOI in the treated waters. It is important to note that half of the TOI samples for site A were below the MDL, so it is unclear whether this significance is conclusive. Overall, these results first show that TOI was present in the raw water. This observation is probably due to the reduction of iodate by soil organic matter. This produces reactive intermediate species (HOI and elemental iodine (I_2)) that undergo rapid electrophilic substitution reactions with electron donor groups on the NOM leading to the formation of organic iodine species (Francois, 1987a; Fukui et al., 1996; Steinberg et al., 2008b; Whitehead, 1974b). Recently, Bowley et al. (2016) showed that both iodide and iodate react with humic acid (a fraction of NOM) in soils to produce organic iodine. They also showed that the humic

acid itself originally contained native sources of iodine, namely iodide and organic iodine. The presence of TOI in raw water due to these reactions is distinctive compared to TOBr and TOCl formation since the latter two do not form in the absence of chloramines and bromamines (Bichsel and von Gunten, 1999). The second observation was that TOI does not significantly increase or decrease upon treatment of the raw water samples. Given that previous research supports that I-DBPs form upon chloramination, the lack of change in TOI concentration between the raw and treated water suggest that the components of TOI may have shifted. That is, after treatment, there is a change in speciation in the contents of TOI from non-DBP iodinated organics to I-DBPs. If this holds true, it underscores the limitation of using a surrogate measure such as TOI since the components of the iodinated organics are not individually identified.

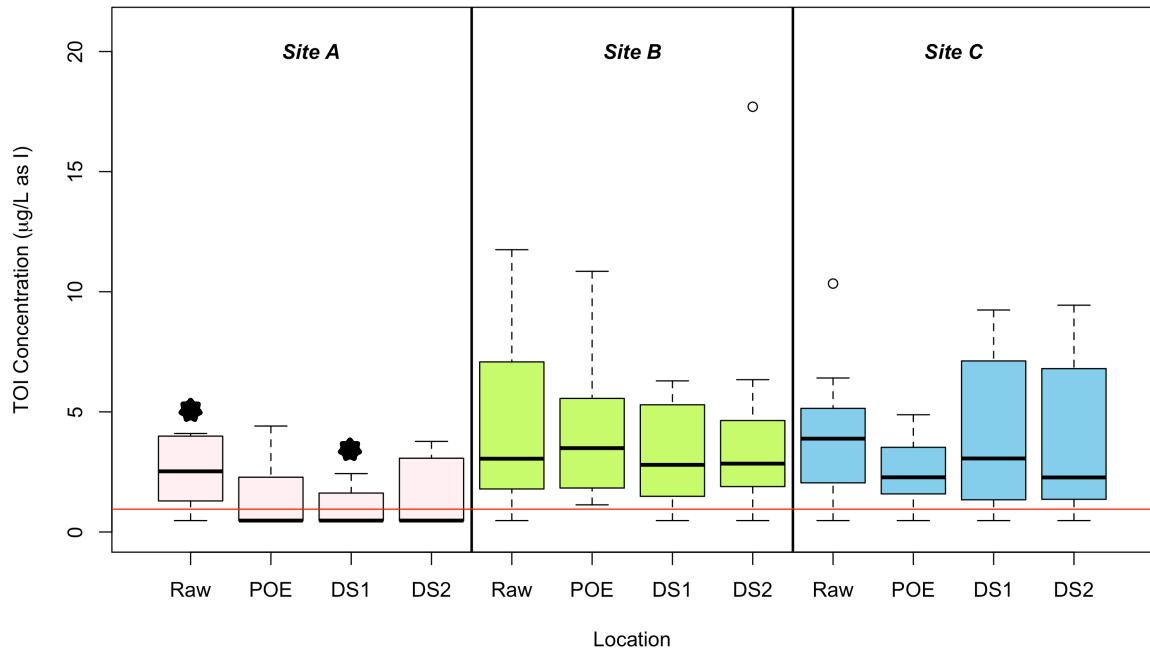


Figure 3.4: Boxplot of TOI concentration at every sampling location pooled across the period of 15 months. The horizontal red line represents the MDL of 0.95 µg/L. TOI values that were below the MDL (n=35) were assumed as half the MDL (0.475 µg/L). The black star indicates significant difference between the locations (p-value < 0.05).

We also explored the concentration of TI and the ratio of TOI to TI across sampling locations to test whether TI or iodine speciation between inorganic and organic species shifts between raw and treated water. The concentration of TI at each of the locations pooled across the months is presented in Figure 3.5. With respect to TI concentration, there was no statistically significant difference between the mean TI concentration in the raw water and that in every other treated sampling point at any of the three sites ($p\text{-value} > 0.05$). However, despite the ANOVA results, raw waters for sites A and C do exhibit somewhat lower TI concentrations compared to the treated waters, particularly in the upper and lower quartiles of the distribution.

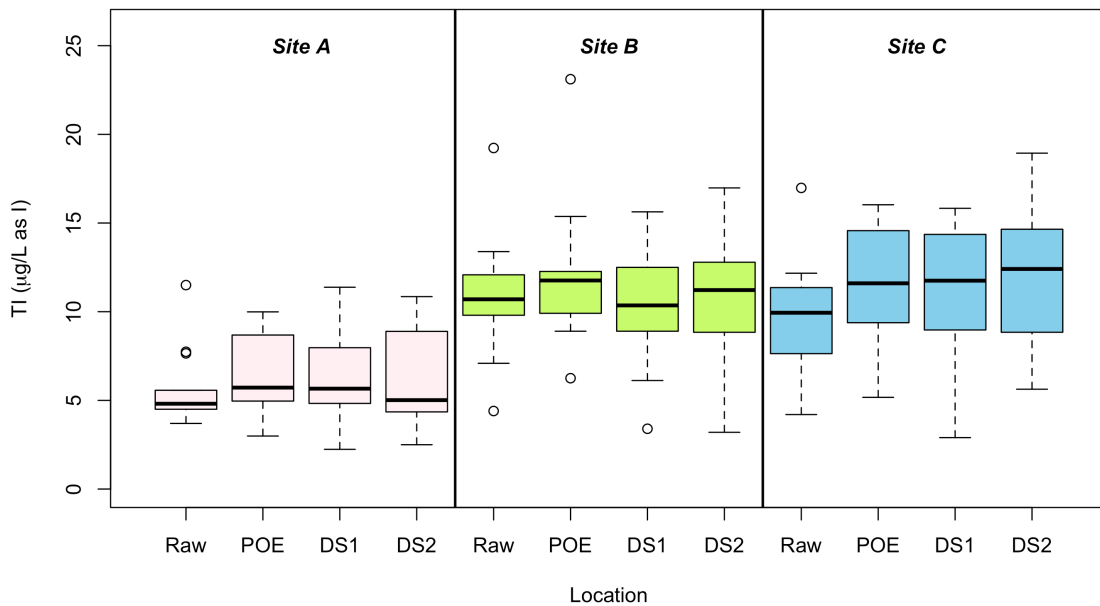


Figure 3.5: Boxplot of TI concentration at every sampling point pooled across the period of 15 months.

For the ratio of TOI to TI, a significant change between raw and treated waters is seen for site A, where the ratio of iodinated organics decreases significantly upon treatment ($p\text{-value} < 0.005$; Fig. 3.6). For example, the median ratio drops from 0.5 in Raw A to about 0.1 in the treated samples. A similar decreasing signal is seen in site C

and is near significant ($p\text{-value} = 0.13$), while no significant difference is seen for site B. The pooled results of the ratio of TOI to TI across all three sites indicate that the ratio of TOI/TI decreases upon treatment ($p\text{-value} < 0.01$). The decrease in the organic fraction upon treatment may indicate that during coagulation, flocculation, and settling processes within the WTP, some of the NOM that is bound to iodine is removed.

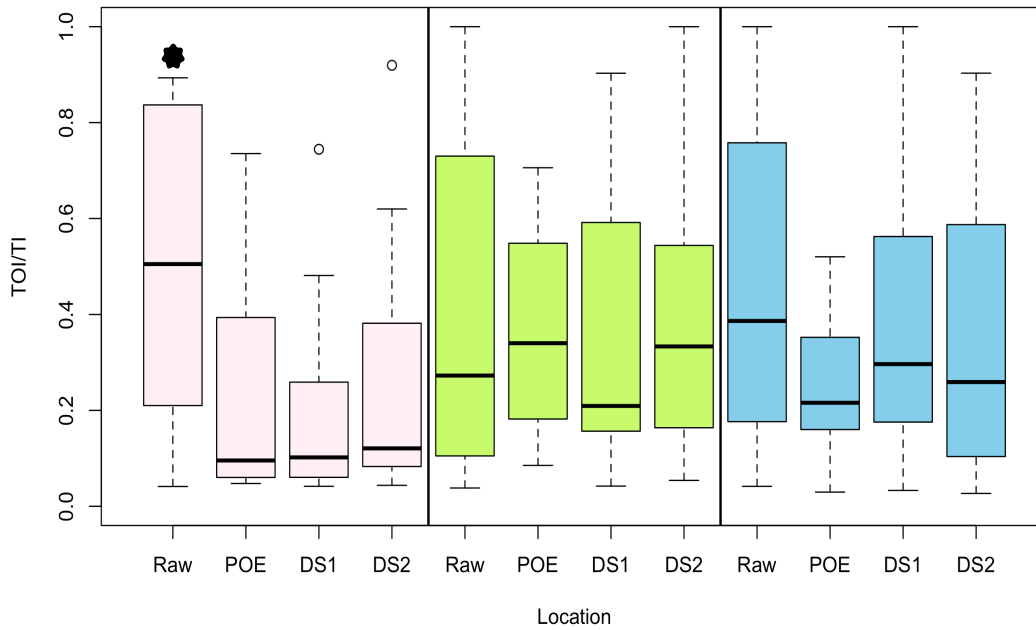


Figure 3.6: Boxplot of TOI/TI at every sampling point pooled across the period of 15 months. Ratios of TOI/TI that were slightly greater than 100% (see Section 3.2.6.2) were forced to 100% for clarity. The black star indicates significant difference between the locations ($p\text{-value} < 0.05$).

3.3.3. Relationship with commonly measured parameters

Given the skewness in TOI concentrations seen in Figure 3.2, the TOI concentration data was log transformed prior to running the Tobit regression. This transformation produced more normally distributed residuals, which are needed for the interpretation of the significance tests for the regression parameters. The results of the Tobit regression are presented in Table 3.7. Of the eight chosen parameters (TI, T, pH,

TCl₂, DOC, NH₃-N, SUVA₂₅₄, and chloride), four exhibited a significant relationship with TOI (p-value < 0.05). DOC and SUVA were highly significant (p-value < 0.0001 and p-value < 0.005, respectively) with opposing trends since they are inversely proportional to one another, while TCl₂ and pH were significant at the 0.05 level. Scatterplots of each of the individual predictors against the measured TOI values are presented in Figure 3.7.

The observed positive relationship between DOC and TOI is expected since upon chloramination of iodide-containing waters, HOI forms (Bichsel and von Gunten, 1999) which in turn reacts with NOM to form I-DBPs (Bichsel and von Gunten, 2000; Richardson et al., 2008; Hua and Reckhow, 2006; Karpel Vel Leitner et al., 1998). Allard et al. (2015) showed that at low DOC concentrations (1 mg/L), almost all of the iodide in the water is converted to iodate, the stable form of iodine. In contrast, at high DOC concentrations (4 mg/L), iodine binds to the many reactive sites within the NOM, forming iodinated organics before it can be converted to iodate. In general, the authors showed that at high DOC concentrations, a higher formation of iodinated organic compounds is expected. This is in line with what we observed in our study.

SUVA₂₅₄ values indicate the nature and composition of NOM in a water sample (Edzwald and Tobiasson, 1999) and have been shown to play a role in the type of DBP formation. Higher SUVA₂₅₄ values (~4 or greater) imply high hydrophobicity, aromaticity, and molecular weight components, while low SUVA₂₅₄ values (<2) indicate the opposite. SUVA₂₅₄ values between 2 and 4 suggest mixtures of hydrophobic and hydrophilic, and aromatic and non-aromatic NOM with a mixture of molecular weights. During the 15 months, we observed SUVA₂₅₄ values that were between 1.85 - 4.17 for the

treated waters (Appendix, Table A.4). Chloramination experiments conducted by Kristiana et al. (2009) showed that the aromatic components within NOM played a major role in the formation of TOBr and TOCl, but not in the formation of TOI. The authors suggested that when the number of reactive sites in NOM have a high aromatic content (high SUVA₂₅₄ values), hypobromous and hypochlorous acids (known to be highly reactive with phenolic moieties), outcompete HOI in reactions with NOM (Criquet et al., 2015; Echigo and Minear, 2006; Lee et al., 2005; Westerhoff et al., 2004). This fact could explain what we observed here: for the low to mid-range SUVA₂₅₄ values observed in this study (1.85 - 4.17), TOI concentration decreases with an increase in SUVA₂₅₄. Allard et al. (2015) observed similar results in chloraminated water, but attributed this to the chlorination/bromination of the active sites during the chlorine contact period.

After treatment, the pH of the samples was adjusted to between 6.93 and 7.45 prior to release into the distribution system throughout the 15 months and across all three sites (Appendix, Table A.4). The effective pH range for monochloramine is between 7.5 and 9. The further the pH drops below 7.5, monochloramine becomes less stable and is likely to dissociate into HOCl and NH₃ (Deborde and von Gunten, 2008). In other words, decreasing the pH is expected to enhance the release of HOCl by the hydrolysis of NH₂Cl. Since HOCl rapidly oxidizes HOI to iodate (the stable form) (Bichsel and von Gunten, 1999; Bichsel and von Gunten, 2000), it leads to lower formation of I-DBPs. This is consistent with what we observed in our study, where an increase in pH was accompanied by a subsequent increase in TOI concentration.

The effect of TCl₂ on TOI concentration is examined next. Our results show that an increase in TCl₂ concentration is accompanied by a decrease in TOI concentration.

This could be seen as a result of an equilibrium between chloramine and free chlorine achieved at sufficiently high TCI_2 concentrations. Once equilibrium is achieved, HOCl starts slowly forming, which then leads to the rapid oxidation of HOI to form iodate (Bichsel and von Gunten, 1999; Bichsel and von Gunten, 2000). This has been previously hypothesized in the literature (Hua et al., 2006) and concurs with the negative effect of TCI_2 on TOI observed in the regression.

Table 3.7: Results of the Tobit regression model.

	Coefficient estimate	Standard error	t value	p-value	Significance
TI	-0.03	0.03	-1.09	0.27392	
T	0.00	0.02	-0.12	0.90492	
pH	1.77	0.88	2.00	0.04517	*
TCI_2	-0.65	0.26	-2.51	0.01207	*
DOC	2.60	0.57	4.56	0.00001	***
$\text{NH}_3\text{-N}$	0.65	2.24	0.29	0.77229	
SUVA	1.02	0.34	2.99	0.00281	**
Chloride	0.00	0.00	0.45	0.65038	
Intercept	-16.71	6.08	-2.75	0.00596	**

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

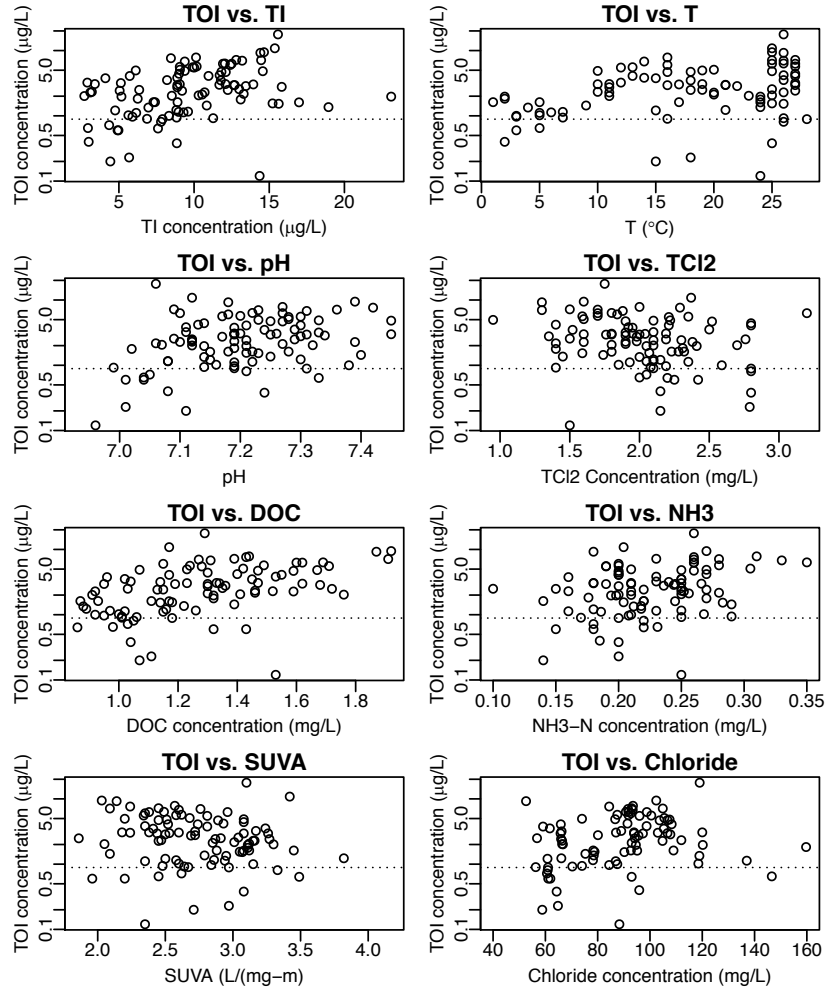


Figure 3.7: Relationships between TOI and the eight chosen predictors. The TOI concentration is on a log scale. The horizontal dotted line indicates the MDL.

The Tobit regression was refit using only the four significant parameters (DOC, SUVA_{254} , TCl_2 , and pH) to develop a predictive model for TOI (Table 3.8). The performance of the model against the observed (measured) values of TOI is presented in Figure 3.8. The model generally provides a good fit ($R^2=0.46$, $p\text{-value} < 0.001$). In order to calculate this R^2 , we assigned a value of half the MDL ($0.475 \mu\text{g/L}$) to the twenty-eight values that were below the MDL. We acknowledge that there is a high variability in the data whereby, for example, an observed value of $20 \mu\text{g/L}$ corresponds to a predicted

value of 2 $\mu\text{g/L}$. This emphasizes the need to improve the current model to minimize such errors in prediction.

Table 3.8: Results of the final Tobit regression model.

	Coefficient estimate	Standard error	t value	p-value	Significance
pH	2.22	0.75	2.95	0.003177	**
TCl₂	-0.44	0.20	-2.24	0.024997	*
DOC	2.06	0.39	5.32	1.05e-07	***
SUVA	0.60	0.24	2.51	0.011951	*
Intercept	-18.63	5.36	-3.47	0.000521	***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

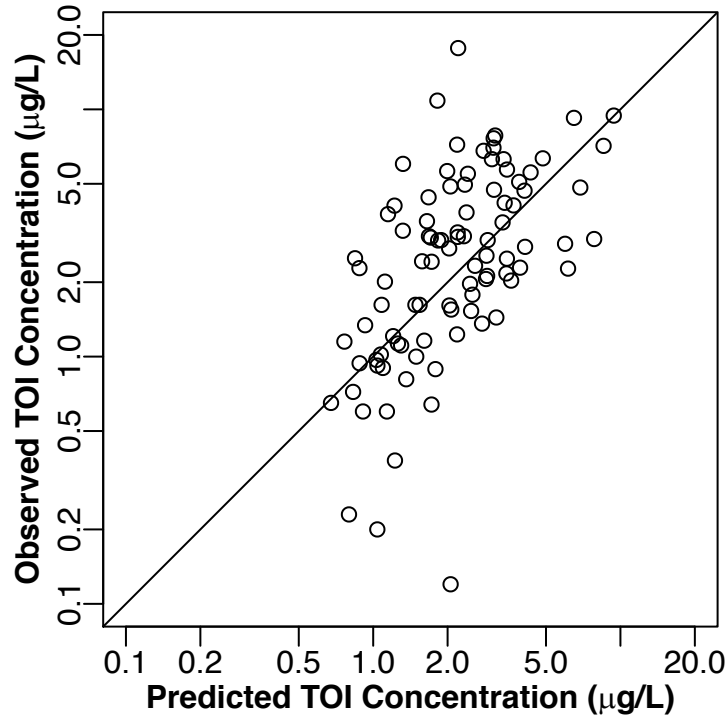


Figure 3.8: Predicted versus observed TOI concentration. The covariates used predict the TOI concentration are DOC, SUVA, pH, and TCl₂. Note: Both x and y axes are on a log scale. The R² for the final Tobit model is 0.46 and the p-value was less than 0.001.

Although the Tobit model determined the previous four parameters (DOC, SUVA₂₅₄, pH, and TCl₂) as the most significant, this does not definitively rule out the influence of other parameters on TOI. For instance, as TI concentrations increase in

water, it is more likely that TOI concentrations will increase as well, mainly due to the higher availability of iodine to form TOI. In fact, iodinated species such as organic iodine, iodate, and iodide are labile and can undergo transformation into one another in the environment (Amachi et al., 2005a; Councell et al., 1997; Farrenkopf et al., 1997; Gong and Zhang, 2013; Radlinger and Heumann, 2000). Our results showed that TOI concentrations did increase with an increase in TI concentrations (Figure 3.6) even though the regression coefficient was not significant ($p\text{-value} > 0.05$). The positive relationship is expected since an increase in TI is expected to be coupled with an increase in all three species (iodide, iodate, and organic iodine). The lack of significance could be due to remaining multicollinearity between the covariates that confounds significance testing. Also, some high TI values at sites B and C during June of 2014 were not accompanied by similarly high TOI values (Appendix, Fig. A.2) which led to a weakening in the expected signal.

Given that TI can be informative in assessing TOI concentration in the water, it is worthwhile to examine variations of TI in the water. One aspect of interest is how TI varies with other halogens, particularly TBr, due to their similar chemical behavior. Upon pooling of all the results, the relationship between the TI and TBr shows that an increase in TI is accompanied by a similar increase in TBr ($R^2=0.45$) (Fig. 3.9). This would suggest that the heavier halogens vary together and could cause joint variations in iodinated and brominated DBPs that are of concern to regulators and utility managers.

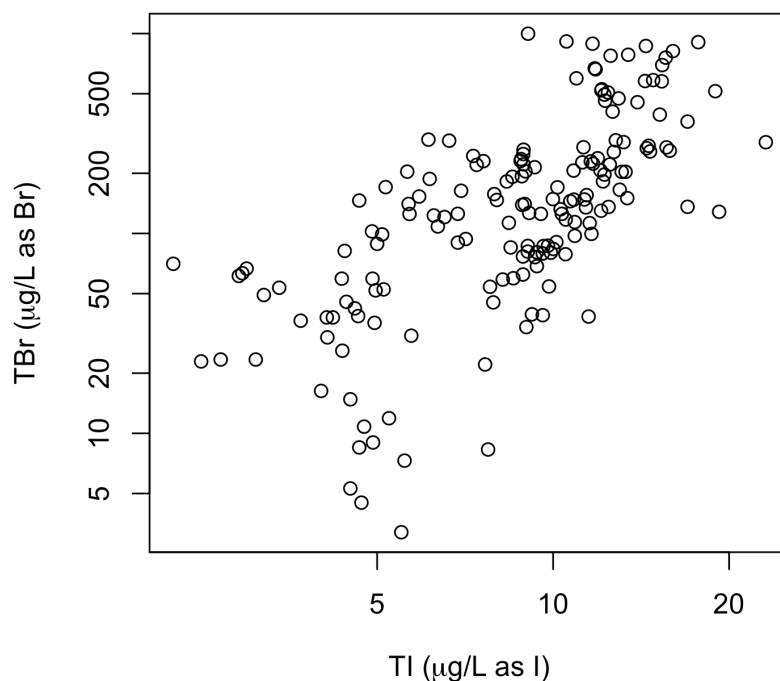


Figure 3.9: Relationship between TI and TBr. Note: Both x and y axes are on a log scale. The R^2 for the linear regression between these two (log-transformed) parameters was 0.45.

3.4. Implications for water treatment

The work presented here characterizes TOI and TI in three drinking water treatment trains in the Northeast US. We have established the order of magnitude of both species over a period of 15 months as well as the differences in the raw water and several points in the distribution system. We observed a clear increase in TI during the spring and summer months compared to the fall and winter months. Despite the absence of a clear seasonal pattern for TOI, there was substantial inter-monthly variability where TOI values cluster high or low for months at a time. The TOI concentrations exhibited peak values between the late summer and early winter. The results showed that TOI was present in the raw water and that there was no change in TOI concentration upon treatment, suggesting that the TOI components may have shifted between the raw and

treated waters. Although the concentration of TOI and TI did not significantly change between raw and treated water, the ratio of TOI to TI was somewhat lower in the treated raw water than in the raw water, potentially due to removal of NOM during the treatment process.

The results of our multivariate regression showed that TOI was correlated with DOC, SUVA₂₅₄, TCl₂, and pH to varying degrees. TOI increased with an increase in DOC and pH and a decrease in SUVA₂₅₄ and TCl₂. A predictive model that used these four parameters was fit for TOI occurrence in water and was able to explain approximately 46% of the variance of TOI concentrations in the treated waters. This is highly valuable since it will allow the estimation of TOI to a certain degree of certainty in relation to commonly measured parameters in a water sample. We also observed a positive correlation between TOI and TI as well as between TI and TBr. This indicates that the iodine and bromine content could give a broad idea of the expected TOI concentration in a water sample.

The advantages of using a TOI as a surrogate measure is that it can accurately quantify the amount of iodinated organic compounds in a water sample. The drawbacks, on the other hand, resemble the drawbacks of using TOX as a surrogate measure. Namely, the surrogate TOI does not provide an accurate identification of the contents of the water since it does not break down TOI into its specific species. A deeper understanding of TOI and the way it varies with the other constituents in a water sample is required to mitigate this drawback. This can ultimately lead to improved management of the water quality without depending on expensive and complicated analytical techniques and equipment.

CHAPTER 4

COMPARATIVE CYTOTOXICITY OF SIX IODINATED DISINFECTION BY-PRODUCTS ON HEALTHY HUMAN EPITHELIAL COLON CELLS

4.1 Introduction

Chlorination of drinking water is considered one of the most important public health developments of the twentieth century, having decreased water-borne diseases in the United States (CDC, 1999). Microorganisms are effectively inactivated in drinking water through the use of strong oxidants such as chlorine, chlorine dioxide, and chloramines. Non-specific oxidation by these additives to drinking water also oxidize natural organic matter (NOM) and bromide/iodide that are naturally present in source waters, and further yield halogenated disinfection by-products (DBPs). Exposure to DBPs through chlorinated drinking water is a major public health concern given the increased risk of bladder, colon, and rectum cancers (Costet et al., 2011; Villanueva et al., 2004; Bove et al., 2007; Villanueva et al., 2007; King and Marrett, 1996; Koivusalo et al., 1997; Morris et al., 1992; McGeehin et al., 1993; King et al., 2000; Rahman et al., 2010; Bull et al. 1995; Cantor et al., 2010).

Within the context of water quality, the ultimate goal of public health agencies is to ensure that communities are provided with safe drinking water. To increase public protection and limit their exposure to DBPs, Stage 1 and Stage 2 Disinfectant and Disinfection Byproduct Rules were imposed by the US Environmental Protection Agency (USEPA) leading to the regulation of 11 DBPs (EPA, 1998, 2003). Following the introduction of the Stage 2 Disinfectant and Disinfection Byproduct Rule, many water utilities in the US shifted from the use of free chlorine to the use of chloramine as a final

disinfectant in an effort to decrease the formation of regulated DBPs (Seidel et al., 2005; USEPA, 2012; Krasner et al., 1989; Zhang et al., 2000). One of the major drawbacks of the use of chloramine as a final disinfectant is that monochloramine, the primary active disinfectant produced during chloramination, reacts slowly with hypiodous acid (HOI) and this favors a slow reaction between HOI with NOM to form iodinated disinfection by-products (I-DBPs) (Bichsel and von Gunten, 1999, 2000). Table 1 shows the twenty compounds that are currently identified as I-DBPs in chlorinated and chloraminated drinking waters. Of those twenty compounds, fifteen have been detected in chlorinated and chloraminated waters (Bichsel and von Gunten, 1999; Brass et al., 1977; Cancho et al., 2000; Chu et al., 2012; Glaze et al., 1975; Jeong et al., 2015; Krasner et al., 2006; Plewa et al., 2004a; Plewa et al., 2008; Richardson, 2003; Weinberg et al., 2002). In simulated chloraminated drinking water, a few hundred I-DBPs were identified (Ding and Zhang, 2009; Wang et al., 2016). About half of the I-DBPs reported by Wang et al. (2016) had aromatic structure, which had been shown to be substantially more toxic than aliphatic I-DBPs (Pan et al., 2016a). Furthermore, I-DBPs are of particular concern because of the twenty compounds that have been identified in drinking water, all but one has been implicated with enhanced cyto- and genotoxicity compared to their brominated and chlorinated analogues (Cemeli et al., 2006; Hunter et al., 1996; Jeong et al., 2015; Plewa et al., 2004a; Plewa et al., 2004b; Plewa et al., 2010; Plewa and Wagner, 2009; Richardson et al., 2007; Richardson et al., 2008). Although the scope of this study is cytotoxicity, genotoxicity, or the ability of a chemical to induce DNA mutations, is another measure that is used to assess the toxicity of a chemical. Researchers evaluated both cytotoxicity and genotoxicity and concluded that the toxicity ranking of I-DBPs

compared with other DBPs is often very similar for the two evaluations (Escobar-Hoyes et al., 2013; Pals et al., 2013; Plewa et al., 2004b; Plewa et al., 2008; Plewa et al., 2010). They also concluded that the genotoxicity of haloamides, halonitromethanes, and haloaldehydes is highly correlated with cytotoxicity (Plewa and Wagner, 2009; Plewa et al., 2008; Plewa et al., 2010; Zhang et al., 2010). Therefore, the results of cytotoxicity analysis can extend beyond its traditional meaning to be informative as a toxicity parameter.

Published studies of I-DBPs have primarily evaluated toxicity using Chinese Hamster Ovary (CHO) AS52 cell models. Given that the toxicological response of a chemical will vary between species due to natural heterogeneity, the response in CHO cells cannot necessarily be extended to humans. Therefore, the need to study human cell lines that can be directly extended to the epidemiological evidence such as colon and bladder in the general population is crucial. Across the twenty I-DBPs of interest, only iodoacetic acid has been the subject of cytotoxicity evaluation in human cell lines (Table 4.1), of which only two used healthy human cells (Attene-Ramos et al., 2010; Escobar-Hoyes et al., 2013). Given that I-DBPs in drinking water are of public health concern, it is important to further assess the toxicity of other I-DBPs. In this study, we chose to evaluate the cytotoxicity of I-DBPs on a non-transformed human colon cell line. Since previous CHO studies have demonstrated that haloamides, a group of nitrogenous-DBPs, exhibit higher toxicity compared to haloacids, a group of non-nitrogenous DBPs (Krasner et al., 2006; Plewa et al., 2008; Plewa and Wagner, 2009), we chose a panel of six iodoacids and iodoamides to allow for relative comparisons between the carbonaceous and nitrogenous groups and between the different cell lines.

Table 4.1: Occurrence of I-DBPs in treated water, the cytotoxicity models used, and information inferred from cytotoxicity assays

Group/Compound	Occurrence in drinking water*	Model	Lowest cytotoxic concentrati on (µM)	LC ₅₀	Reference
Iodoacid					
Iodoacetic Acid	+ ^{a, b}	<i>S. typhimurium</i>	150	~250	Cemeli et al., 2006
			100	303	Plewa et al., 2004b
		CHO-AS52 cells	0.5	4	Cemeli et al., 2006
			0.5	2.95	Plewa et al., 2004b
		CHO-K1 cells	1.68	5.3	Zhang et al., 2010
		TK6 cells	35	80	Hilliard et al., 1998**
		FHs cells	22	NA	Attene-Ramos et al., 2010
		Caco-2 cells	NA	34	Procházka et al., 2015
		HepG2 cells, rat hepatocytes, and intact rats	2	8	Wang et al., 2014
		NIH3T3 cells	2.5	2.7	Wei et al., 2013
Primary human lymphocytes	NA	NA	Escobar-Hoyes et al., 2013		
Diiodoacetic acid	-	CHO-AS52 cells	100	332	Richardson et al., 2008
Bromiodoacetic acid	+ ^{a, b}	CHO-AS52 cells	250	897	Richardson et al., 2008
(Z)-3-bromo-3-iodopropenoic acid	+ ^{a, b}	CHO-AS52 cells	75	208	Richardson et al., 2008
(E)-3-bromo-3-iodopropenoic acid	+ ^{a, b}	CHO-AS52 cells	25	145	Richardson et al., 2008
(E)-3-bromo-2-iodopropenoic acid	-	CHO-AS52 cells	17.5	436	Richardson et al., 2008
(E)-2-iodo-3-methylbutenedioic acid	+ ^{a, b}	CHO-AS52 cells	700	944	Richardson et al., 2008
Iodomethane					
Dibromiodomethane	+ ^c	CHO-AS52 cells	1500	1900	Richardson et al., 2008
Dichloriodomethane	+ ^c	CHO-AS52 cells	2000	4130	Richardson et al., 2008
Bromochloriodomethane	+ ^c	CHO-AS52 cells	2200	2400	Richardson et al., 2008
Bromodiodomethane	+ ^c	CHO-AS52 cells	1500	NA	Richardson et al., 2008
Chlorodiodomethane	+ ^c	CHO-AS52 cells	1000	2410	Richardson et al., 2008
Iodoform	+ ^c	CHO-AS52 cells	10	66	Richardson et al., 2008
		NIH3T3 cells	55	83.4	Wei et al., 2013
Iodonitrile					
Iodoacetnitrile	-	CHO-AS52 cells	0.1	3.3	Muellner et al., 2007
Iodoamide					
Iodoacetamide	-	CHO-AS52 cells	0.5	1.42	Plewa et al., 2008
		LLC-PK1	10	NA	Chen and Stevens, 1991
Diiodoacetamide	-	CHO-AS52 cells	0.025	0.678	Plewa et al., 2008
Bromiodoacetamide	+ ^{d,e}	CHO-AS52 cells	2	3.81	Plewa et al., 2008
Chloriodoacetamide	+ ^e	CHO-AS52 cells	2	5.97	Plewa et al., 2008
Iodoaldehyde					
Iodobutanal	+ ^{a,c}	NA	NA	NA	NA
Iodoacetaldehyde	+ ^f	NA	NA	NA	NA

*: indicates positive occurrence (+) and negative occurrence (-); ^a: Krasner et al., 2006; ^b: Plewa et al., 2004b; ^c: Weinberg et al., 2002; ^d: Plewa et al., 2008; ^e: Chu et al., 2012; ^f: Jeong et al., 2015. ** indicates that sodium iodoacetate was used

4.2. Materials and Methods

4.2.1. Reagents and chemicals

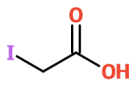
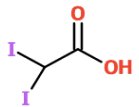
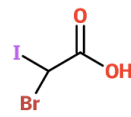
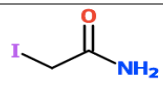
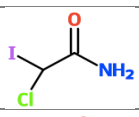
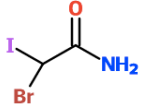
Dulbecco's Phosphate Buffer Saline (D-PBS; 30-2200TM stored at 4 °C), Eagle's Minimum Essential Medium (EMEM; 30-2003TM stored at 4 °C), Penicillin-Streptomycin Solution (30-2300TM stored at -20 °C), dimethylsulfoxide (DMSO, 4-XTM stored at 4 °C), Trypsin-EDTA Solution (1X, 30-2101TM stored at -20 °C), and fetal bovine serum (FBS) (30-2020TM stored at -20 °C) were purchased from American Type Culture Collection (ATCC). PrestoBlue® Cell Viability Reagent was obtained from ThermoFisher Scientific and stored at 4 °C. Diiodoacetic acid (DIAA, >90%), bromoiodoacetic acid (BIAA, >85%), bromoiodoacetamide (BIAcAm, >85%), and chloroiodoacetamide (CIAcAm, >99%) were purchased from CanSyn Chemical Corporation (Toronto, Canada). Iodoacetamide (IAcAm, >99%) and iodoacetic acid (IAA, >98%) were purchased from Sigma-Aldrich. Triton® X-100 (Molecular Biology Grade) was obtained from Promega.

4.2.2. Preparation of solutions

Three iodoacids and three iodoamides were prepared to be tested for cytotoxicity (Table 4.1). IAA stock solution was prepared by dissolving IAA in EMEM solution containing 10% FBS and 1% Penicillin-Streptomycin. For the other five compounds, stock solutions were prepared by dissolving each compound in EMEM containing 10% FBS, 1% Penicillin-Streptomycin, and 0.1% DMSO. The prepared EMEM solutions containing each of the six I-DBPs had a pH between 7 and 7.5 and were used within 4 weeks. The I-DBPs were assumed to be stable within that range of pH and timeline.

Preliminary tests were conducted for each compound to determine the range of concentrations to be used for cytotoxicity analysis. Serial dilutions in the appropriate media were used to make a total of 6 concentrations for each compound to be analyzed for cytotoxicity (Table 4.1). All reported concentrations are in $\mu\text{g/L}$ as I. All solutions were stored at 4 °C and used within four weeks. The cells were cultured in EMEM containing 10% FBS and 1% Penicillin-Streptomycin (EMEM-C).

Table 4.2: Summary of the concentration ranges for each of the six I-DBPs

Compound	Molecular structure	Abbreviation	Chemical formula	Molecular weight (g/mol)
Iodoacetic acid		IAA	$\text{C}_2\text{H}_3\text{IO}_2$	185.95
Diiodoacetic acid		DIAA	$\text{C}_2\text{H}_2\text{I}_2\text{O}_2$	311.84
Bromoiodoacetic acid		BIAA	$\text{C}_2\text{H}_2\text{BrIO}_2$	264.84
Iodoacetamide		IACAm	$\text{C}_2\text{H}_4\text{INO}$	184.96
Chloroiodoacetamide		CIACAm	$\text{C}_2\text{H}_3\text{ClINO}$	219.41
Bromoiodoacetamide		BIAcAm	$\text{C}_2\text{H}_3\text{BrINO}$	263.86

4.2.3. Cell culture

Immortalized (non-neoplastic) normal human colon epithelial cells, CCD 841 CoN (CRL-1790), were obtained from ATCC at passage 13 and were used in all experiments between passage 15 and 17. The CCD 841 CoN cells were isolated from a 21-week gestation fetus that did not show any abnormalities (Thompson et al., 1985). The

cells were cultured and maintained in T75 tissue culture flasks with EMEM-C containing 10% non-heat inactivated FBS and 1% Penicillin-Streptomycin Solution at 37 °C in a humidified 5% CO₂ incubator. Cells were maintained until 80% confluence before subsequent assays described below.

4.2.4. Human cell cytotoxicity assay

CCD 841 CoN cells were seeded (12,500 cells per well) in clear, sterile, 96-well microplates with EMEM-C media and cultured at 37 °C in a humidified 5% CO₂ incubator. Upon reaching 80% confluence, the media was replaced with fresh media containing different concentrations of the six compounds (exposure media), EMEM-C (positive control for IAA), or EMEM-C with 0.1% DMSO (positive control for five other compounds). The plates were then incubated for 12 hours at 37 °C in the humidified 5% CO₂ incubator. At hour 11, Triton® X-100 (1 µL) was added to the positive controls and incubated for one hour. Following the exposure period, cells were washed with D-PBS. EMEM-C (90 µL) and PrestoBlue (10 µL) were added to each well and incubated for 1 hour at 37 °C in the humidified 5% CO₂ incubator. A SpectraMax® MiniMax™ Imaging Cytometer (Molecular Devices) was used to measure the fluorescence at 535 nm for excitation and 615 nm for emission at 52 points per well. For each compound, 2 to 5 biological replicates were tested at each concentration. Exposures were repeated on 3 or 4 separate days (experimental replicates), yielding a total of 9 to 15 replicates per concentration per compound. Cell viability was evaluated as the number of viable cells while cell cytotoxicity was evaluated as the reduction in the number of viable cells compared to the positive control.

4.2.5. Data analysis

Cytotoxicity data was normalized to the averaged percent of the corresponding negative controls from individual experiments. The average mean viability values obtained from the biological and experimental replicates from all the experiments were used to construct a cell viability concentration-response curve. The data from each compound were used to generate Four-Parameter Logistic nonlinear regression functions using the “log(inhibitor) vs. response” equation (Eq. 4.1).

$$Y = Bottom + (Top - Bottom)/(1 + 10^{(LogIC_{50}-X) \times HillSlope}) \quad \text{Eq. 4.1}$$

where Y is the percent of cells that are viable, X is the tested concentration, and HillSlope is the slope of the sigmoidal curve. The Top and Bottom values were constrained to 100 (all cells are viable) and 0 (all cells are not viable), respectively.

To fit the curve for each compound, the parameters were adjusted to minimize the mean square error between the fitted values and observations. The root mean squared error was then calculated for each of the fitted curves to reflect every compound according to Eq. 4.2.

$$R^2 = 1 - \frac{RSS}{TSS} = 1 - \frac{\sum_{j=1}^n (y_{j,i} - \hat{y}_{j,i})^2}{\sum_{j=1}^n (y_{j,i} - \bar{y}_i)^2} \quad \text{Eq. 4.2}$$

where RSS and TSS stand for residual sum of squares and total sum of squares, respectively, n is the number of tested concentrations, j is the number of data points from 1 to n, i is the number of compounds, y, \hat{y} , and \bar{y} are the observed, estimated, and average viability across the tested concentrations, respectively.

The optimized curves were used to calculate the LC_{50} values, or the lowest concentration at which 50% reduction in cell density is observed as compared to control cells. For each LC_{50} value, a mean cytotoxicity index value was calculated as $1000/LC_{50}$ as previously described by Jeong et al. (2015) such that a larger value corresponds to higher cytotoxicity. Both LC_{50} and the cytotoxicity index can be used to rank the cytotoxicity of the six I-DBPs for this particular cell line. Another measure of interest is the lowest cytotoxic concentration, which was determined as the lowest concentration that induced a significant reduction in cell density as compared to the negative control. The significance was tested using a one-way analysis of variance (ANOVA) and a Tukey's post-hoc test (p -value < 0.05). All data were analyzed using the programming language *R*.

4.3. Results and discussion

An immortalized normal epithelial-like colon cell (CCD 841 CoN) line was selected given the positive association reported by epidemiological studies between exposure to chlorinated water and increased risk of colon cancer. The CCD 841CoN cells were exposed to three iodoacids (IAA, BIAA, and DIAA) and three iodoamides (IAcAm, BIAcAm, and CIAcAm) at varying concentrations (Table 4.2) and were analyzed for acute cytotoxicity.

4.3.1. CCD 841 CoN cytotoxicity

Figure 4.1 presents a dose-response curve for the six DBPs. The viability of CCD 841 CoN cells was found to decrease in a concentration-dependent manner within the tested range of concentrations for the six compounds. Cytotoxicity was evaluated using

two parameters: lowest cytotoxic concentration at which there was a statistically significant decrease in viable cells compared to the negative control and LC_{50} (Table 4.3). The lowest concentration that was found to induce to a cytotoxic response compared to the controls ranged was 8 μM for IAA, 25 μM for IAcAm, 50 μM for BIACAm, 100 μM for both CIACAM and DIAA, and 1000 μM for BIAA (p-values < 0.05). The LC_{50} values ranged from 8.6 μM for IAA to about 1 mM for DIAA and BIAA. In CCD 841 CoN cells, the rank order for cytotoxicity of the six I-DBP compounds based on their LC_{50} values was as follows: IAA > IAcAm > BIACAm > CIACAM > DIAA \approx BIAA.

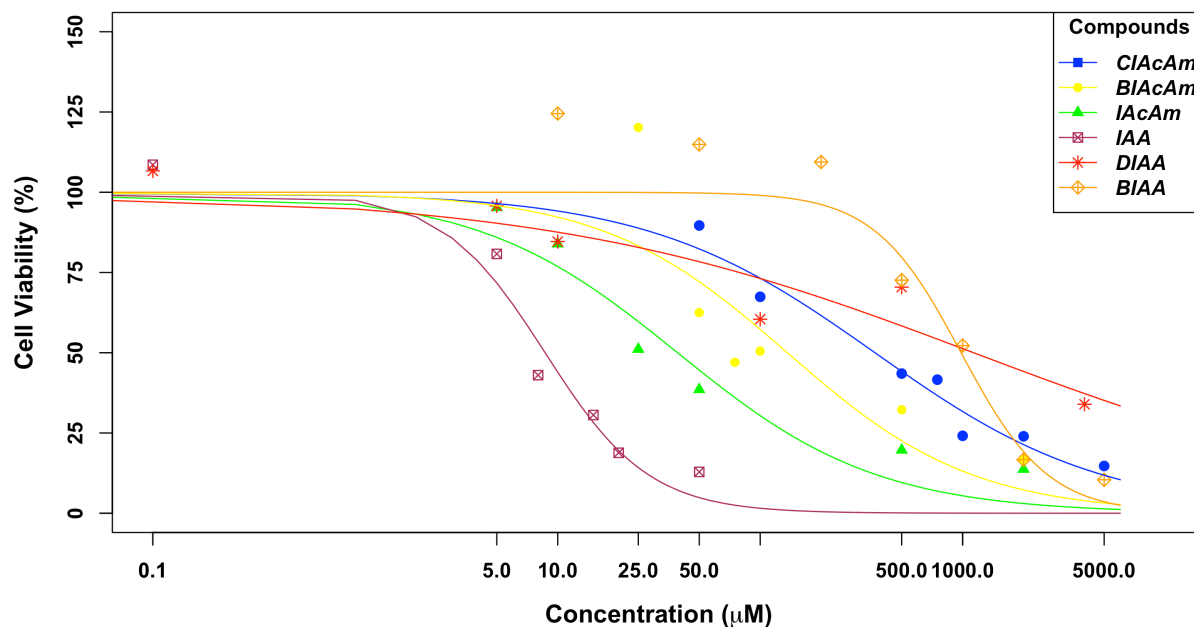


Figure 4.1: Concentration-response curves of the six I-DBPs on CCD 841 CoN cells.

Table 4.3: Summary of the CCD 841 CoN cell cytotoxicity of the I-DBPs

I-DBP	Tested Concentration range (μM)	Lowest cytotoxic concentration ^a (μM)	ANOVA p-value ^b	LC ₅₀ ^c (μM)	R ² ^d	Toxic rank order
IAA	0.1 - 50	8	0	8.6	0.83	1
IACAm	5 - 2000	25	0.0003	39.1	0.76	2
BIACAm	25 - 2000	50	0.0008	136.3	0.52	3
CIACAm	50 - 5000	100	0.0171	369.0	0.69	4
DIAA	0.1 - 4000	100	0.0017	954.7	0.51	5
BIAA	10 - 5000	1000	0.0269	982.2	0.81	6

^aThe lowest cytotoxic concentrations at which a chemical induced a significant cytotoxicity as compared to the negative control determined by ANOVA test.

^bThe results of the ANOVA for the lowest cytotoxic concentration

^cThe concentration at which the cell viability was reduced by 50% as compared to the negative control determined by the non-linear Four-Parameter Logistic nonlinear regression functions.

^dThe root mean squared error for the non-linear curve fitting

A cytotoxicity index value, calculated from LC₅₀, was evaluated as another strategy to rank the tested compounds with a higher number corresponding to higher cytotoxicity. Figure 4.2 shows the six I-DBPs ranked on the basis of their cytotoxicity index value. Of the six iodinated compounds (IAA, BIAA, DIAA, IACAm, BIACAm, and CIACAm), IAA exhibited the highest cytotoxicity to CCD 841 CoN cells. This finding is in line with published literature which reports enhanced cytotoxicity for IAA compared with other haloacetic acids in CHO-AS52 cells (Richardson et al., 2008). The increased cytotoxicity of IAA has been attributed to the length of the carbon-halogen bond and the number of halogens per atom. As the size of the halogen increases, the dissociation energy declines making iodine an excellent leaving group compared to the other halogens; this consequently leads to higher cytotoxicity (Plewa et al., 2004b). The pattern of decreasing toxicity of I>Br>Cl has been observed for all identified I-DBPs except for iodoacetaldehyde (Jeong et al., 2015). Enhanced toxic potency is also attributable to fewer halogens are per atom (Plewa et al., 2002). However, previous studies showed that the haloacids was less cytotoxic than the haloamides in CHO-AS52 cells (Plewa et al., 2008, Plewa et al., 2010). These results were consistent with the results of the di-

halogenated but not for the mono-halogenated compounds in this study: IAA was four and a half times more cytotoxic than IAcAm in CCD 841 CoN cells. This may indicate that human colon cells are more sensitive to IAA than IAcAm. Since haloamides were not explored in other cell lines than CHO cells, the significance is unclear.

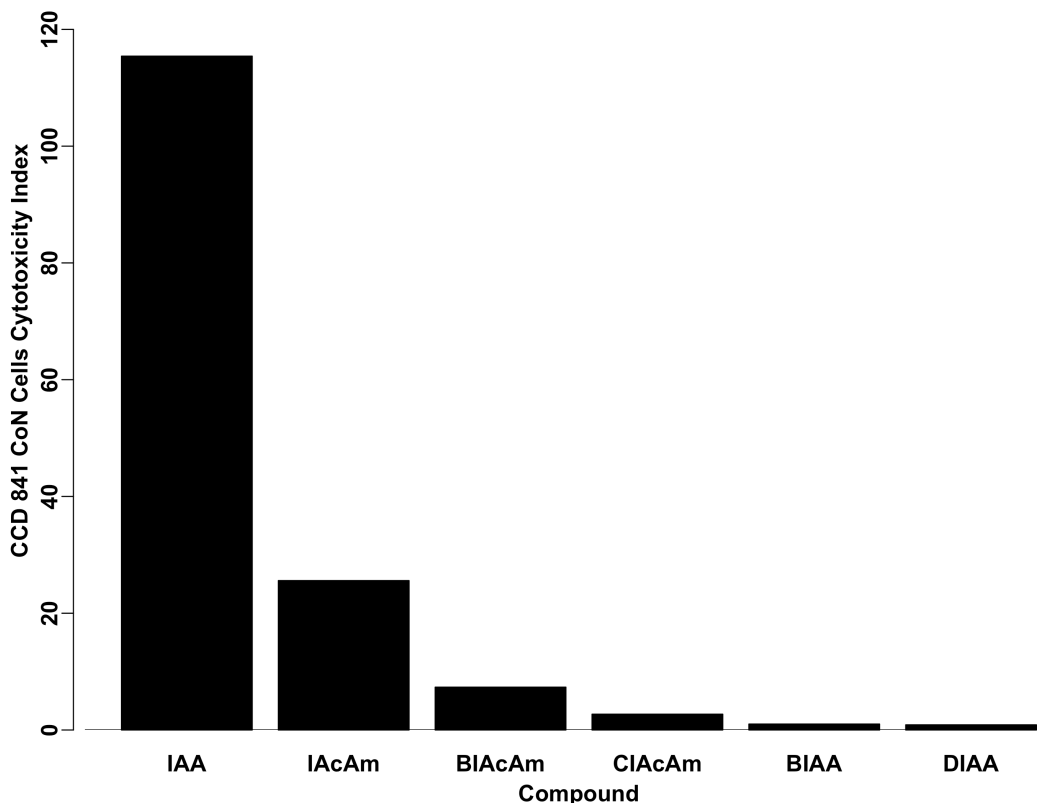


Figure 4.2: Comparison of the CCD 841 CoN cells cytotoxicity index values ($LC_{50}/1000$) of the tested I-DBPs. The higher the cytotoxicity index value, the more cytotoxic the compound.

The lowest cytotoxic concentration for IAA in CCD 841 CoN cells was 8 μ M, corresponding to about 1000 μ g/L. The maximum IAA concentration in water utilities has been reported at 1.7 μ g/L in the US (Richardson et al., 2008) and 2.18 μ g/L in China (Wei et al., 2013). The lowest cytotoxic concentration of BIAA was 1000 μ M. This is 10 times greater than that of DIAA. However, the LC_{50} , and consequently the cytotoxicity

index value of DIAA and BIAA was about the same. The cytotoxicity of both dihaloacids (DIAA and BIAA) was less than 1% of that of the monohaloacid IAA. This indicates that an increase in halogens per atom was associated with a decrease in the toxic potency of DBPs as previously observed in CHO-AS52 cells (Plewa et al., 2002). BIAA has been found in chloraminated water across water utilities in the United States, however, concentrations have not been reported in the literature (Krasner et al., 2006; Plewa et al., 2004b; Richardson et al., 2008). DIAA has very been detected and quantified in two treated samples at very low concentrations (2.2 and 3.3 ng/L) in China (Pan et al., 2016). Although the detected concentrations of IAA are many orders of magnitude lower than the lowest cytotoxic concentration, the health outcomes associated with long-term exposure may influence the long-term health impacts.

IAcAm was 3.5 times more cytotoxic than BIAcAm, which in turn was 2.7 times more cytotoxic than CIAcAm to CCD 841 CoN cells. A similar trend was also observed in CHO-AS52 cells with respect to monohaloamide cytotoxicity, reinforcing the relationship between halogen size on dissociation energy and toxicity (Plewa et al., 2002; Plewa et al., 2008). BIAcAm has been detected in the sub- to low- $\mu\text{g/L}$ levels in three raw water and nine treated waters around the US (Plewa et al., 2008) and in 3 WTPs in China (Chu et al., 2012). These reported levels are below the lowest cytotoxic concentration of 50 μM (6.3 mg/L) observed in this study. CIAcAm has not been detected in water utilities in the US but has been detected in treated samples from three urban water treatment plants in China at concentrations ranging from 80 and 210 ng/L (Chu et al., 2012). The detected values are orders of magnitude below the lowest cytotoxic concentration reported for CIAcAm reported here which was about 12.7 mg/L

(100 μ M). The DBP, IAcAm has not been detected in either raw or treated water samples (Chu et al., 2012; Plewa et al., 2008).

It is worth noting that one of the drawbacks of any *in vitro* cytotoxicity assay to a cell line is that the impact of a toxicant cannot be unequivocally extrapolated to the same cell line within humans. This is since the direct exposure of a toxicant to a cell line is vastly different from human exposure via ingestion or inhalation, for example. Therefore, the effects between the two to the toxicant will not be identical.

4.3.2. Comparative cytotoxicity of CCD 841 CoN cells and other cell lines

Differences across cytotoxicity assays, as well as inherent sensitivity across cell lines to particular compounds, will affect the overall response of a cell line to a compound (Procházka et al., 2015). Although it is not ideal to relate the sensitivity of human cells with mouse or hamster cells because of their characteristic and species specific differences, a comparison of the toxicity rank of different cell lines to a toxicant could potentially offer some useful insight to these cell lines. The only compound that we can do such a comparison on across all I-DBPs is IAA since it has received the most attention.

Figure 4.3 compares the cytotoxicity of IAA using a variety of mammalian cells, including CHO (AS52 and K1), mouse (NIH3T3), and human (HepG2 and CCD 841 CoN) cells. NIH3T3 cells, CHO-AS52 cells, and CHO-K1 cells were exposed to IAA for 72 hours (Cemeli et al., 2006; Plewa et al., 2004b; Wei et al., 2014; Zhang et al., 2010). With regards to human cells, HepG2 cells were exposed over a 24-hour period to IAA (Wang et al., 2014) while CCD 841 CoN cells in this study were exposed for 12 hours. The results demonstrate comparable cytotoxicity between the human cell models (CCD

841 CoN cells from this study and HepG2 liver cells) (Wang et al., 2014). Compared to cell lines of mouse and Chinese hamster origin (CHO-AS52, CHO-K1, and NIH3T3), CCD 841 CoN cells were 2 to 3 times less sensitive to IAA (Cemeli et al., 2006; Plewa et al., 2004b; Wei et al., 2014; Zhang et al., 2014). Since the size of the mammal directly influences its metabolic rate due to anatomic, physiologic, and biochemical differences (Perlman, 2016; Schmidt-Nielsen, 1984), the difference in sensitivity could be explained by differences in size between small animals (e.g., mice and hamsters) and humans.

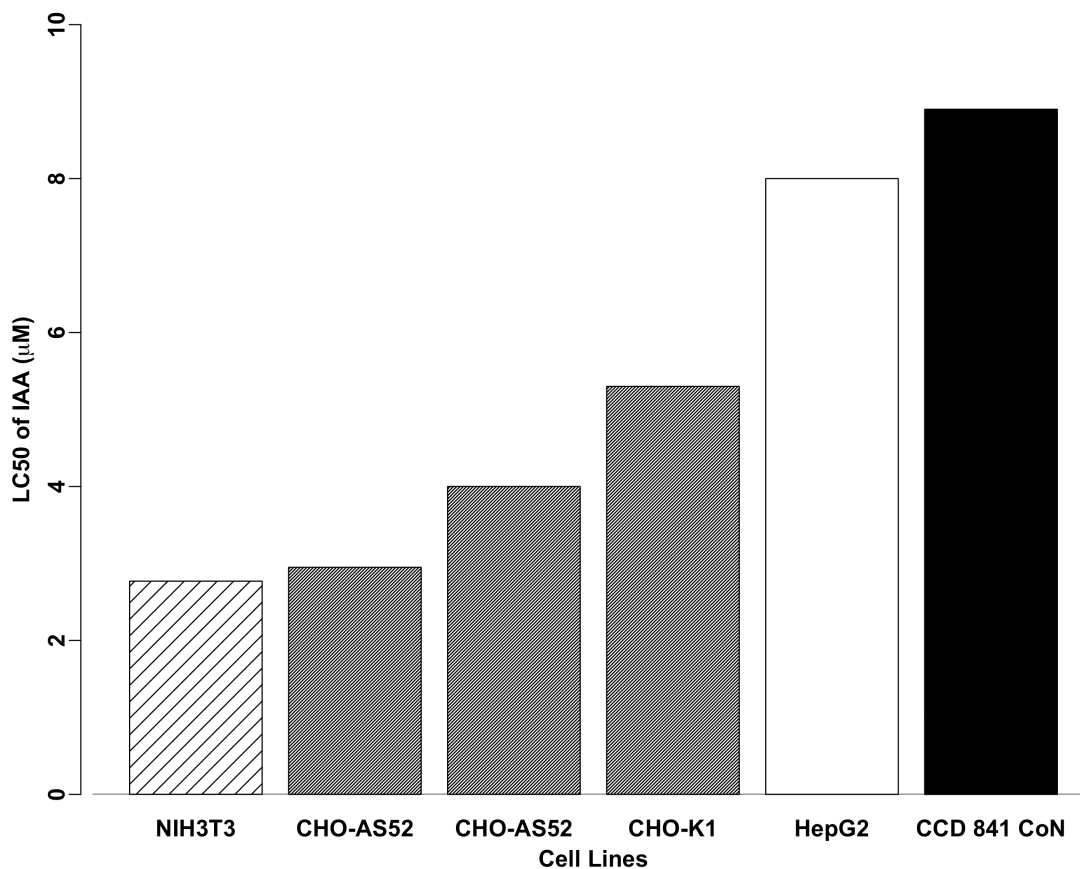


Figure 4.3: Comparison of the LC₅₀ calculated from the cytotoxicity of IAA on different mammalian cell lines from the literature (Cemeli et al., 2006; Plewa et al., 2004b; Zhang et al., 2010; Wang et al., 2014; Wei et al., 2013) and the present study. The striped bar (NIH3T3) indicates a mouse embryo cell line. The grey bars (CHO) indicate Chinese hamster cell lines. The white bar (HepG2) indicates a human liver cell line. The black bar (CCD 841 CoN) indicates the human colon cell line used in this study. Note: the CCD 841 CoN cell exposure time was 12 hours compared to 24 hours for the HepG2 cells and 72 hours for the NIH3T3 cells, CHO-AS52 cells, and CHO-K1 cells.

The cytotoxicity of the other five compounds (DIAA, BIAA, IAcAm, BIACAm, and CIACAm) have previously only been tested using CHO-AS52 cells (Plewa et al., 2008; Richardson et al., 2008). Figure 4.4 presents a comparison of the LC₅₀ between the five compounds testing CCD 841 CoN cells used in the current study and CHO-AS52 cells from the published literature. The exposure period for CHO-AS52 cells was 72 hours compared to 12 hours in our study. The exposure concentration ranges were

between 1 and 1000 μM for DIAA, 100 and 2500 μM for BIAA, between 0.5 and 2.5 μM for IAcAm, between 2 and 10 μM for BIAcAm, and between 2 and 100 μM for CIAcAm for CHO-AS52 cells (Plewa et al., 2008; Richardson et al., 2008). The range of concentrations evaluated in our study extend beyond those evaluated in CHO-AS52 cells (Table 4.2). The three iodoamides were associated with lower LC_{50} values (39.1 - 369 μM) compared to the dihaloacids (~ 1000 μM) indicating that apart from IAA, nitrogenous-DBPs were more cytotoxic than carbonaceous-DBPs. The trend across DBPs for both cell lines was found to be similar. The results from our study shows that the sensitivity of CCD 841 CoN cells to these five compounds was lower than those reported in the literature despite the shorter exposure period and higher exposure concentrations (Richardson et al., 2008; Plewa et al., 2008). This may indicate a higher resilience of human colon cells to these toxicants than CHO cells, leading to the decrease in observed sensitivity.

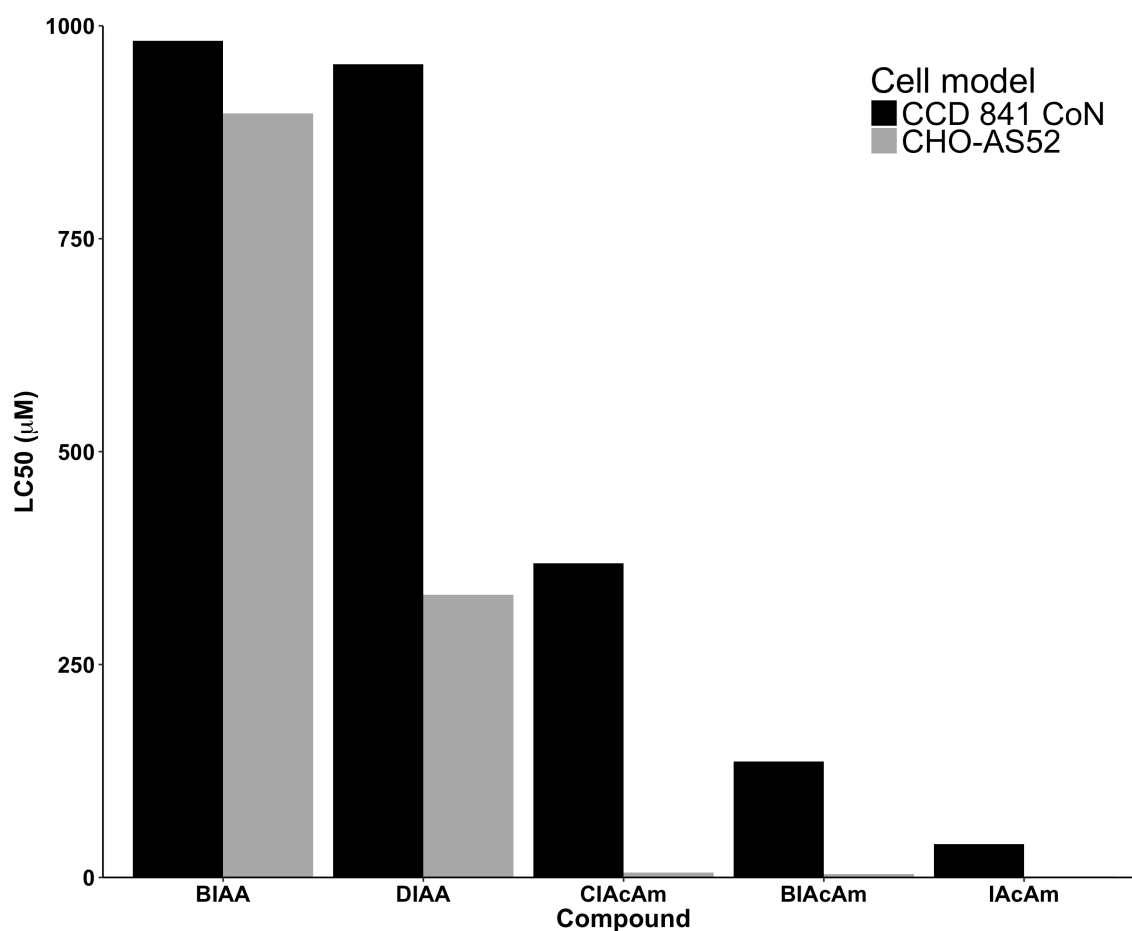


Figure 4.4: Comparison of the LC₅₀ between five I-DBPs on CCD 841 CoN cells (this study) and CHO-AS52 cells (Richardson et al., 2008; Plewa et al., 2008).

4.3. Applications to water treatment systems

The three tested iodoacids (IAA, BIAA, and DIAA) have been identified and/or quantified in real (non-simulated or modified) drinking water. In a nationwide DBP occurrence study of the US, it was shown that IAA and BIAA formation was most prevalent and the highest in plants that use chloramine as a final disinfectant (Richardson et al., 2008). This follows previous studies in the literature that show that chloramination increases I-DBP formation compared to chlorination (Bichsel and von Gunten, 1999; Bichsel and von Gunten, 2000; Krasner et al., 2006). For iodoacids, several other factors

play a role in the extent of formation. For example, iodide levels (due to salt water intrusion) in the raw water of two rivers in China caused an increase in IAA concentration in the finished (chloraminated) water (Wei et al., 2013). The same was also observed in the DBP occurrence study of the US (Richardson et al., 2008). Furthermore, the occurrence study showed that WTPs that have a short chlorine contact time prior to chloramine addition (<1 min), or those where chlorine and ammonia are added simultaneously, form higher concentrations of iodoacids (namely, IAA and BIAA). The use of a long chlorine contact time (>45 min) would tend to decrease the formation of iodoacids, however it may cause an undesirable increase in regulated HAA and THM formation that originally motivated the shift from chlorination to chloramination.

As a class, iodoamides have been shown to be about 100 times more cytotoxic compared to HAAs in CHO cells (Plewa et al., 2008). We showed that apart from IAA, the three tested iodoamides were more cytotoxic in CCD 841 CoN cells than the haloacids. Of the three tested iodoamides in this study, two (BIAcAm and CIAcAm) have been identified and quantified in real chlorinated and chloraminated drinking water. In the nationwide occurrence study, BIAcAm was found in the raw and treated water of three WTPs as well as in the treated water of nine other WTPs (Plewa et al., 2008). All twelve WTPs had a naturally high iodide and bromide content and used chloramine as a final disinfectant. Chu et al. (2012) also identified and measured both CIAcAm and BIAcAm in 3 WTPs in China either due to chlorination or chloramination. IAcAm was the most cytotoxic of the three iodoamides in our study but it has yet to be identified in treated waters (Chu et al., 2012; Plewa et al., 2008). Haloacids have been extensively studied in the literature since they were first identified as a DBP. Similar efforts need to

be put forth for the newly identified nitrogenous I-DBPs to develop strategies for controlling their formation.

This study suggests that IAA and the three iodoamides (IAcAm, BIAcAm, and CIAcAm) should be prioritized given their enhanced cytotoxicity observed in a human cell line. Further studies need to be conducted to understand the specific factors that lead to their formation in drinking water while also meeting the DBP regulatory standards. It would be extremely useful to monitor the concentration of TOI as well as some of the identified priority I-DBPs in actual water treatment plants and their distribution system to assess how they vary together. This could give a higher utility to the use of TOI as a surrogate for I-DBPs.

CHAPTER 5

CONCLUSIONS

TOI is a convenient aggregate measure of I-DBPs in disinfected water. A new TOI method was developed that builds on previous methods while overcoming their drawbacks. The method entailed adsorbing a highly acidified sample ($\text{pH} < 1$) onto two granular activated carbon columns, combustion of the two columns to release HI gas that gets trapped in a basic ($\text{pH} \sim 12$) water solution. The solution is then analyzed using the ICP-MS with a wash solution of TMAH (0.1% v/v.) This method effectively recovered a wide range of iodinated organics. Given the method's low detection limit, we were also able to successfully measure TOI in raw and treated water at very low concentrations.

Upon developing this TOI method, we were able to investigate the presence of ambient TOI in raw and treated waters. We established the order of magnitude and the patterns of variation of TOI, as well as the contribution of TOI to TI in multiple raw water locations, water treatment sites, and distribution systems. By doing so, we created a novel data set of TOI. One of the original objectives of this work was to develop predictive models for TOI in treated water, as measuring TOI requires advanced analytical instrumentation. Having a comprehensive data set enabled us to do this by exploring the correlations between TOI and routinely measured parameters in treated water. DOC, SUVA, TCI_2 , and pH were all significantly related to TOI concentration to varying degrees and explained about half of the variance of TOI concentration. This work shows the potential of predicting TOI in treated water based on its physical and chemical parameters.

Since most toxicity studies of I-DBPs have been conducted on CHO cells, there was a need to study human cell lines that can be directly extended to the epidemiological evidence such as colon and bladder cancer in the general population. Therefore, we evaluated the cytotoxicity of six I-DBPs (three iodoacids and three iodoamides) on a non-transformed human colon cell line. Several toxicity parameters enabled us to study the response of the human colon cell line to different tested I-DBPs and subsequently rank their cytotoxicity. Our study suggests that IAA and the three iodoamides tested here should be prioritized with regards to preventing their formation given their enhanced cytotoxicity observed in a human cell line. In order for these advances to be useful for management of public health, we need to ensure that variations in TOI also reflect variations in the I-DBPs that are of greatest health concern. Therefore, future work is needed to measure the variations of these I-DBPs in water systems with respect to variations in TOI.

Future studies are also needed to characterize the toxicity of I-DBPs and to develop toxicity libraries among other halogenated DBPs using the human colon cell line. This includes expanding the cytotoxicity assessment to include the other haloacids and haloamides that were not covered in this study, as well as the I-DBPs from other iodinated groups that have been identified in treated waters. In parallel, a comparative cytotoxicity assessment with brominated and chlorinated DBPs from those same groups should be conducted. This will allow for a creation of a new ranking system of DBP toxicity by group and by compound to complement the existing CHO one. Furthermore, studies are also needed to evaluate the cellular and molecular mechanisms associated with I-DBPs since they are not well understood. A toxicogenomic study is one approach

that can be very informative in analyzing changes in gene expression in the CCD 841 CoN cell line due to exposure to I-DBPs. It would provide insight on the mode of action of the toxicants and on the gene expression profiles related to adverse impacts such as oxidative stress, inflammation, cancer, and changes in metabolism. The ultimate hope is that this cell culture can be used as a prototype of human colon tissue to simulate the response of human cells to exposure to I-DBPs.

SYMBOLS AND ABBREVIATIONS

I^- : iodide
 IO_3^- : iodate
 I_2 : elemental iodine
 $NaIO_3$: sodium iodate
ANOVA: analysis of variance
ATCC: American Type Culture Collection
BIAA: bromoiodoacetic acid
BIACAm: bromoiodoacetamide
CCD 841 CoN: Human non-transformed (healthy) epithelial colon cells
CHO: Chinese Hamster Ovary cells
CIACAm: chloroiodoacetamide
D-PBS: Dulbecco's Phosphate Buffer Saline
D/DBP: disinfectant and disinfection byproduct
DBPs: disinfection by-products
DIAA: diiodoacetic acid
DMSO: dimethylsulfoxide
DOC: dissolved organic carbon
DS: distribution system
EMEM-C: Eagle's Minimum Essential Medium-Complete
EMEM: Eagle's Minimum Essential Medium
GAC: granular activated carbon
HI: hydrogen iodide
 HNO_3 : nitric acid
HOI: hypoiodous acid
I-DBPs: iodinated disinfection by-products
I-THMs: iodinated trihalomethanes
IAA: iodoacetic acid
IACAm: iodoacetamide
IC: Ion Chromatography
ICM: iodine x-ray contrast media
ICP-MS: Inductively Coupled Plasma – Mass Spectrometer
KBr: potassium bromide
KI: potassium iodide
MDL: method detection limit
NaI: sodium iodide
 NH_3 -N: ammonia-nitrogen
 NO_2 -N: nitrite-nitrogen
 NO_3 -N: nitrate-nitrogen
NOM: natural organic matter
POE: point of entry
SUVA: specific UV absorbance
TBr: total bromine
 TCl_2 : combined chlorine residual
TI: total iodine

TII: total inorganic iodine
TMAH: tetramethyl ammonium hydroxide
TOBr: total organic bromine
TOC: total organic carbon
TOCl: total organic chlorine
TOI: total organic iodine
TOX: total organic halogen
WTP: water treatment plant

APPENDIX SUPPORTING INFORMATION

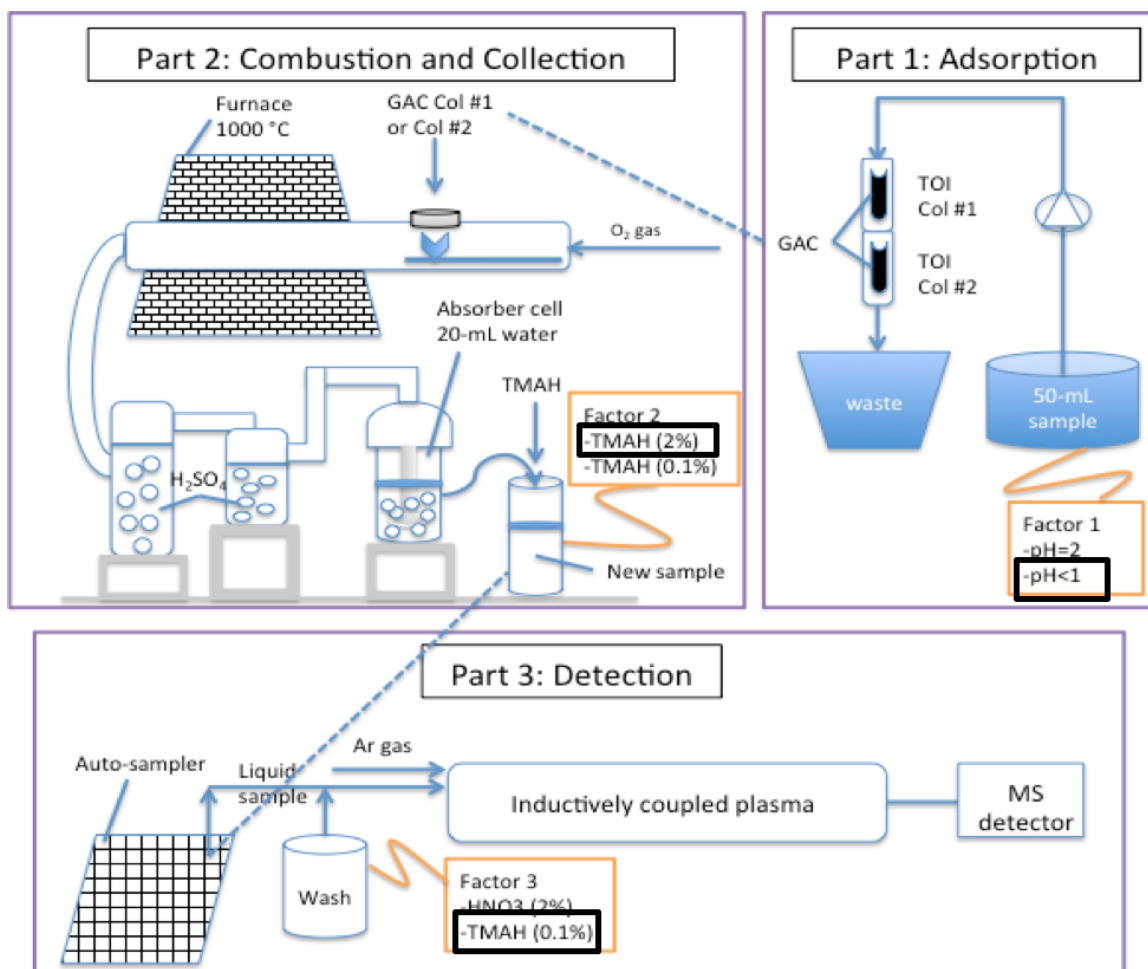


Figure A.1: Graphical representation of the chosen TOI method

Table A.1: Average physical and chemical characteristics of the real water samples over the two summer months

Index	Type of Water	¹ pH	T (°C)	Conductivity (μS/cm)	TOC (mg/L)	DOC (mg/L)	Residual Chlorine (mg/L)	UV ₂₅₄ (1/cm)	SUVA ₂₅₄ (L.mg ⁻¹ .m ⁻¹)
A	Raw	7.64	27	241	2.78	2.24	NA	0.079	3.53
A1	Treated	7.26	28	333	1.19	1.18	2.80	0.034	2.85
A2	Treated	7.21	27	335	1.19	1.10	2.53	0.034	3.09
A3	Treated	7.31	27	336	1.14	1.08	2.15	0.032	2.96
B	Raw	7.89	26	548	2.98	2.47	NA	0.073	2.94
B1	Treated	7.20	26	576	1.57	1.46	2.86	0.046	3.16
B2	Treated	7.15	27	534	1.65	1.55	1.67	0.039	2.49
B3	Treated	7.18	26	546	1.53	1.55	1.58	0.042	2.69
C	Raw	7.99	27	488	2.61	2.51	NA	0.069	2.75
C1	Treated	7.32	26	596	1.56	1.49	1.98	0.041	2.76
C2	Treated	7.23	26	592	1.74	1.65	1.60	0.037	2.24
C3	Treated	7.31	26	580	1.63	1.60	1.42	0.037	2.32

¹ at the collection point prior to ascorbic acid addition

Appendix Text - Statistical Analyses

In the ANOVA, the general term $T_{r,s,t}^i$ is used to denote the sum of mean effects for the i^{th} observation associated with the levels for Factors 1, 2, and 3, indexed by r, s and t, respectively (Eq. A.1).

$$T_{r,s,t}^i = \mu + F1_r + F2_s + F3_t + (F1F2)_{rs} + (F1F3)_{rt} + (F2F3)_{st} + (F1F2F3)_{rst} + \varepsilon_{r,s,t}^i \quad \text{Eq. A.1}$$

Here, μ is the factor-independent mean of T ; $F1_r$, $F2_s$, and $F3_t$ are the main effect model terms; $(F1F2)_{rs}$, $(F1F3)_{rt}$, and $(F2F3)_{st}$ are the two-way interaction model terms; $(F1F2F3)_{rst}$ is the three-way interaction model term; and $\varepsilon_{r,s,t}^i$ is the noise/error component.

For the eight organic model compounds, the ANOVA was conducted using the normalized adjusted recoveries by replacing $T_{r,s,t}^i$ with $y_{r,s,t}^i$. The $y_{r,s,t}^i$ are pooled together in the ANOVA for all 8 model compounds and 2 duplicates in order to increase

the statistical power of the test. That is, $i=1, \dots, 16$ observations are available for each *treatment* after pooling. However, the ANOVA tests are repeated for each compound separately to verify the results and explore differences between compounds.

For the inorganic compounds, the ANOVA was conducted by replacing $T_{r,s,t}^i$ with $Rj_{r,s,t}^i$ for each of the low and high concentrations, and for both sets of concentrations pooled together.

Finally, for the real water samples, the ANOVA was conducted upon pooling of the raw and treated waters by replacing $T_{r,s,t}^i$ with the actual concentrations of TOI under different factors. This was done after it was determined that there was an insignificant difference between the raw and treated water samples.

Table A.2: The ANOVA results for the standardized adjusted recovery of the eight compounds for the three factors

Compound	ANOVA p-value		
	Factor 1	Factor 2	Factor 3
IAA	0.007	0.014	2.6e-07
BIAA	0.010	0.041	0.040
TIAA	0.906	0.722	1.7e-06
2-hydroxy-3-iodo-5-nitropyridine	0.853	0.777	0.460
3-iodo-4-methylbenzoic acid	0.130	0.644	0.008
Iopromide	0.219	0.787	0.032
Diatrizoic acid	0.676	0.588	2.2e-07
Iopamidol	0.004	0.081	0.679

Table A.3: The TOI concentration ranges of the field samples (both raw and treated water) without distinction between the variation in the choice of Factors.

Index	Type of Water	Concentration Range ($\mu\text{g/L}$ as I)	
		July	August
A	Raw	b.d.l. - 6.42	2.34 - 4.36
A1	Treated	b.d.l. - 7.89	2.33 - 5.28
A2	Treated	b.d.l. - 5.72	0.69 - 2.53
A3	Treated	1.29 - 3.78	1.42 - 3.64
B	Raw	7.31 - 13.55	5.95 - 11.03
B1	Treated	0.38 - 7.34	5.53 - 6.95
B2	Treated	3.76 - 7.55	4.14 - 6.79
B3	Treated	1.73 - 8.02	4.93 - 6.52
C	Raw	b.d.l. - 5.53	2.45 - 4.89
C1	Treated	0.88 - 6.38	4.32 - 7.41
C2	Treated	2.63 - 7.41	6 - 9.19
C3	Treated	b.d.l. - 7.52	6.01 - 11.91

*b.d.l. indicates a concentration below the method's detection limit.

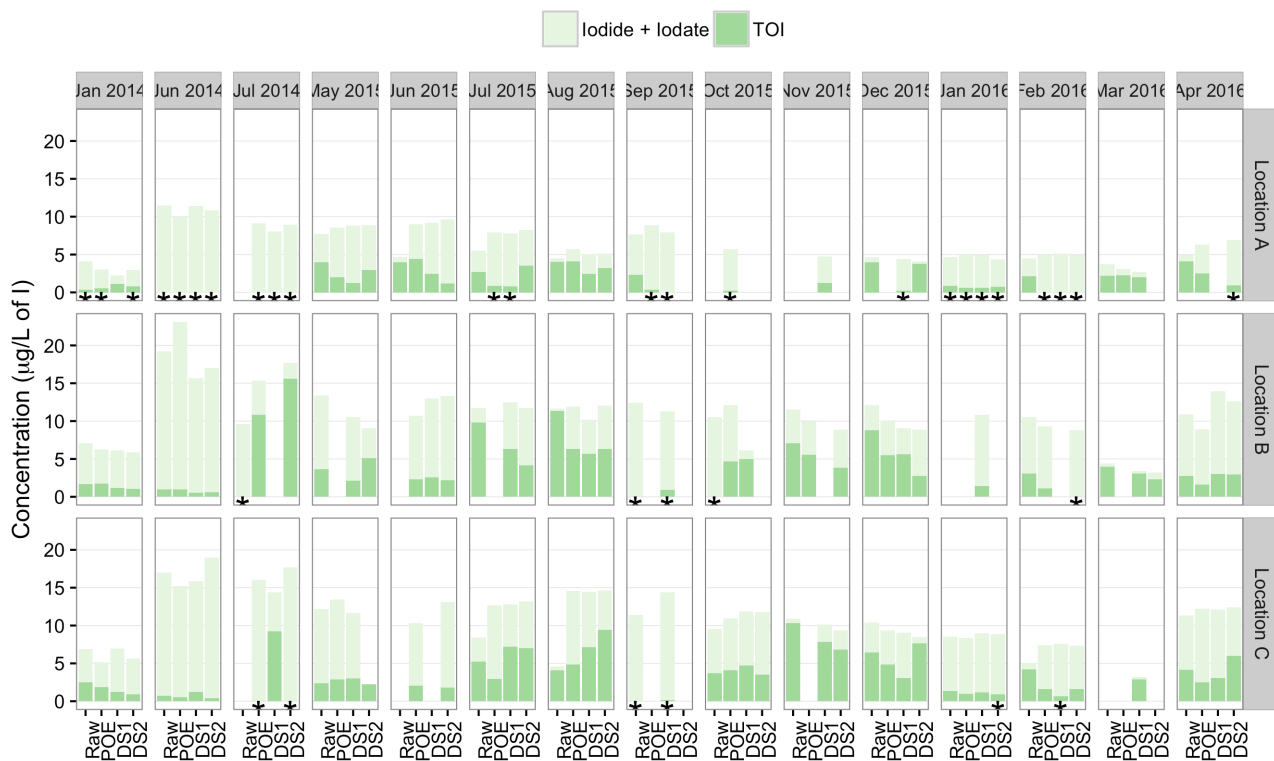


Figure A.2: Concentration of TOI and total inorganic iodine (TII; sum of iodide and iodate) across the twelve locations and 15 months. * represents TOI values that were below the MDL of 0.95, even if a value was measured

Table A.4: Median physical and chemical characteristics of the field samples over the 15 months.

Sample	Type	T range (deg C)	pH range	Conductivity range (μ S/cm)	TOC range (mg/L)	DOC range (mg/L)	Residual chlorine range (mg/L)	UV ₂₅₄ range (1/cm)	SUVA range (L/(mg * m))
A-Raw	Raw	2 - 28	7.15 - 7.94	203 - 290	1.93 - 3.18	1.35 - 2.78	NA	0.065 - 0.113	3.04 - 5.33
A-POE	Treated	2 - 28	7.01 - 7.32	272 - 427	0.95 - 1.34	0.92 - 1.76	2.15 - 2.8	0.026 - 0.04	2.05 - 4.17
A-DS1	Treated	3 - 27	7.02 - 7.3	285 - 362	1.1 - 1.23	0.95 - 1.17	2.1 - 2.8	0.027 - 0.036	1.9 - 3.82
A-DS2	Treated	5 - 27	7.04 - 7.33	294 - 371	0.98 - 1.35	0.94 - 1.26	1.5 - 2.5	0.024 - 0.036	2.44 - 3.26
B-Raw	Raw	2 - 26	7.78 - 8.78	290 - 631	1.57 - 3.21	1.15 - 2.93	NA	0.044 - 0.107	2.46 - 4.38
B-POE	Treated	2 - 26	7.12 - 7.32	433 - 698	1.02 - 1.78	1.02 - 1.71	1.9 - 3.2	0.03 - 0.048	2.34 - 3.42
B-DS1	Treated	5 - 27	6.99 - 7.4	402 - 688	1.06 - 1.7	0.91 - 1.6	0.95 - 2.22	0.027 - 0.041	2.09 - 3.48
B-DS2	Treated	3 - 26	6.93 - 7.28	368 - 640	1.1 - 1.68	0.95 - 1.69	1 - 2.21	0.027 - 0.044	2.35 - 3.1
C-Raw	Raw	2 - 27	7.23 - 9.29	414 - 535	1.5 - 3.85	1.11 - 3.49	NA	0.046 - 0.162	2.39 - 4.64
C-POE	Treated	1 - 27	7.07 - 7.45	486 - 637	0.98 - 1.86	0.92 - 1.72	1.8 - 2.59	0.025 - 0.044	1.86 - 3.24
C-DS1	Treated	4 - 27	6.96 - 7.45	429 - 634	0.89 - 1.95	0.86 - 1.91	1.3 - 2.2	0.025 - 0.041	2.09 - 3.49
C-DS2	Treated	5 - 26	7.02 - 7.42	438 - 616	1.2 - 2.29	0.98 - 1.88	1.2 - 2.29	0.025 - 0.041	1.85 - 3.45

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